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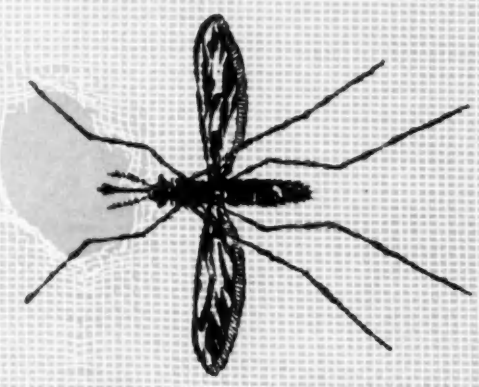

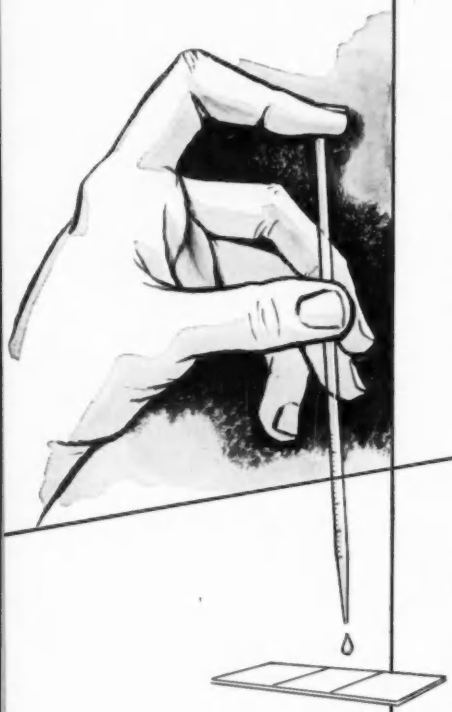

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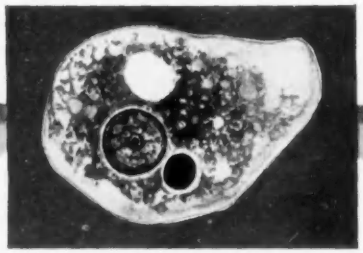

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2. Zavala, D. C., and Hamilton, H. E.: *Ann. Int. Med.*, 36:110, Jan., 1952.
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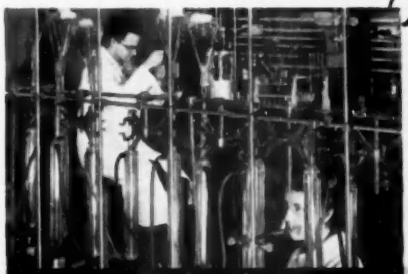

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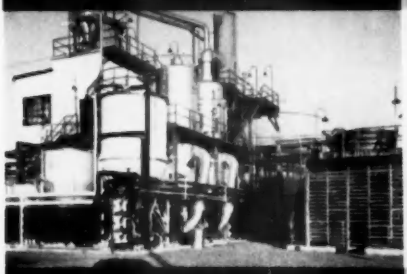

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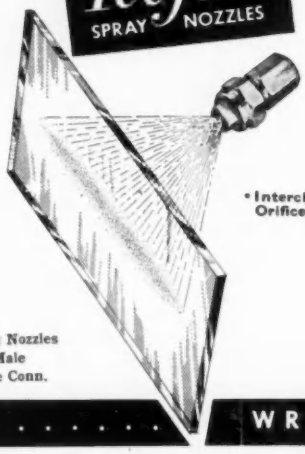

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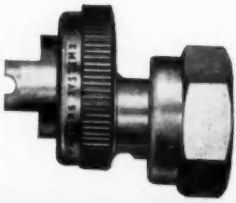

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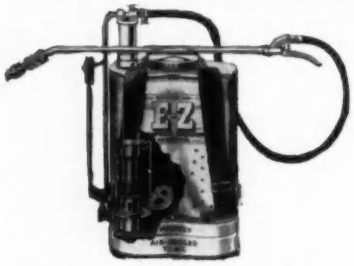

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SOME PROJECTED USES FOR THE AXENIC CULTIVATION OF HELMINTHS[1]

PAUL P. WEINSTEIN

National Institutes of Health, National Institute of Allergy and Infectious Diseases,[2] Bethesda, Maryland

It would be esthetically inappropriate to use the dinner hour as the time to describe the details of the cultivation of parasitic nematodes. Fortunately, the symposium last year gave me the opportunity to present a review of the work, which was subsequently published in the Journal through the kindness of Drs. Hackett and Stoll and, therefore, available to those of you who may be interested in reading it.

I am not sure that what I have to say now will be any more entertaining but if you are in a philosophical mood, it might be appropriate to explore a few intriguing and perplexing problems in helminthology, some aspects of which may be profitably approached using bacteria-free cultivation procedures.

Purely fundamental investigations concerning the conditions necessary for growth, differentiation and reproduction of parasitic helminths in vitro are basic to obtaining the information which will permit the profitable use of cultivation procedures in an applied form. The ultimate goal of such investigations, of course, is the development of synthetic media which will support optimal growth. When such become available for large groups among the nematodes, cestodes and trematodes, new approaches will be possible to problems in physiology and metabolism, immunity, chemotherapy, host-parasite relationships, comparative biology, etc. I would like to discuss now in a general way the projected application of in vitro methods to a few of these areas of research.

The pathogenesis of helminth infections may be ascribed to a multiple number of causes, but for the sake of this brief discussion, I will limit myself to several which are of considerable importance and possess aspects which are particularly suitable for study using in vitro methods; these are sensitization and allergic reactions, damage from toxic substances, metabolic injuries arising from competition for important metabolites, and malignant growths seemingly related to the presence of some parasites.

Probably the outstanding pathognomonic signs characterizing many of the important helminthic infections of man and animals are hypersensitization and allergic response. The various filarial infections, echinococcosis, schistosomiasis, trichinosis, begin a long list of diseases illustrating this. There is considerable evidence accumulated to indicate that the substances primarily involved in sensitization are the secretions and excretions of the worms. Such products have been obtained for study chiefly by grinding and extracting worm material, or by incubating readily obtainable stages of parasites in "survival" media for short periods of time. With suitable cultivation procedures, however, it would be possible to obtain for analysis and experimental work by-products of normally metabolizing worms. In addition, the products of a worm during different stages of growth may well have differences in antigenicity. Once the cycle of development can be obtained in vitro, metabolic and other products of specific stages could be procured with relative ease. Such material would not only be of use in studying the clinical aspects of hypersensitivity, but should aid greatly in developing more specific antigens for diagnostic work.

There is considerable clinical evidence suggesting the elaboration of directly toxic materials by helminths, but experimental data identifying specific substances have been convincing in only a few instances. As in the case of sensitizing substances, in vitro cultures would make available metabolic products for chemical analysis and pharmacological study.

The possibility that a parasite may compete successfully with its host for particular growth factors and that this may result in severe nutritional deficiency of the host is best illustrated by the development of genuine pernicious anemia in some cases of *Diphyllobothrium latum* infection.

[1] Address given at the Dinner Session of the American Society of Tropical Medicine and Hygiene on November 1, 1957, by the recipient of the Bailey K. Ashford Award, provided through the generosity of the Eli Lilly Company.

[2] Laboratory of Tropical Diseases.

The available information indicates that the tapeworm contains a considerable amount of vitamin B_{12} and that under a certain set of circumstances, in competing with the host for the B*12* present in the intestine, it may seriously deplete the amount available to the host. When this occurs over a sufficiently long period of time, pernicious anemia ultimately develops. Similar relations between parasite and host have been claimed for other helminth infections, but definitive data are lacking. Here is an area where the knowledge gained of the specific nutritional requirements of an organism will have much to contribute, especially when a peculiarly high demand for a particular micronutrient may be demonstrated.

There is strong evidence in a few instances that helminth infections (particularly larval *Taenia taeniaformis* in the liver of the rat and *Schistosoma haematobium* in man) are positively correlated with the development of true neoplasms. The underlying mechanisms are as yet unknown. However, the cultivation of these animals in vitro would afford an important tool to investigate the relation of by-products of these organisms to the development of cancerous growths.

I do not think it is necessary to develop at great length the importance of acquiring detailed information about the metabolism and nutritional requirements of a parasite as the underlying basis of a rational approach to chemotherapy Most metabolic studies conducted in the past have been on helminths maintained only in saline solutions, and exhibiting primarily catabolic activity. These systems have been more or less contaminated in many instances with by-products of accompanying bacteria. There is considerable need, in this regard, for the development of appropriate methods of rearing helminths under bacteria-free conditions in nutritive media in which they will show normal metabolic activity. This will insure against errors of misinterpretation due to abnormal metabolic patterns which may develop in "survival"-type media. It will also provide for study particular larval or adult stages against which it may be most logical to direct drugs, but which may be unavailable or available in insufficient amount from in vivo sources.

The understanding of the phenomenon referred to as host specificity and its counterpart, natural resistance, represents one of the most challenging problems facing the helminthologist as well as the general microbiologist today. These terms will have meaning only when the complex biochemical and biophysical reactions which they describe can be subjected to experimental investigation. The study of the host-parasite relation is often complicated by a third factor, i.e., the presence of various microbial associates, as is the case with parasites residing in the gut. Faced with the difficult problem of the interaction of two or more organisms, it would seem logical to know as much as possible about the individuals alone, before or while tackling the extremely difficult study of the interactions between them. In terms of the parasite, intimate understanding will more easily come about when it can be adequately studied in vitro under controlled conditions of cultivation. I, of course, do not mean to imply that by merely assembling a large body of information about the parasite alone and the host alone, it will automatically give us the answers to the many problems involved in the host-parasite relation. It should, however, allow us to pose more intelligent and sophisticated questions of this complex system and in this way perhaps bring enlightenment at a more rapid rate.

The interesting story unfolded by many investigators concerning the role that a particular nutrient may play in determining susceptibility or refractoriness to malaria is apropos at this time. During recent years, information obtained from in vitro cultivation studies, as well as from in vivo investigations, indicated the importance of *p*-aminobenzoic acid as a vitamin for plasmodia. These findings ultimately helped explain the fact that rats, monkeys, and probably man fed on a milk diet exclusively, which is virtually lacking in this vitamin, are refractory to malaria infection. In addition, a rational basis was obtained for understanding the action of various drugs against plasmodia in terms of their antagonism to *p*-aminobenzoic and folic acids.

In the case of helminths having a high degree of host specificity and developing incompletely or not at all in so-called abnormal hosts, how often may this be due similarly to lack of specific nutrients or particular physico-chemical conditions required by the parasite for growth, rather than to such positive factors as cellular response and antibody. This is not to depreciate the importance of the latter, but rather to orient our thinking more often to include the former as well. Our studies with *Nippostrongylus* in vitro have indicated that only partial development equiva-

lent to a late lung stage will occur on chick embryo homogenate alone, and that such worms will survive in an arrested state in excellent condition for a fairly long period of time. Further development to the adult stage takes place, however, when the medium is supplemented with other growth factors. This spectrum of partial to complete development is similarly found to occur in vivo both in nature and in laboratory infections of various abnormal hosts. One may ask whether at least some of the factors influencing such arrested development in vivo may not be attributable to the lack or imbalance of particular nutrients available to the parasite in such abnormal hosts. These hypotheses may be particularly pertinent to the study of the phenomenon of "larva migrans."

Investigations on the mechanism of acquired immunity to helminth infections, particularly to nematodes, have demonstrated a rather complex interaction of serum antibody and cellular response. The net result in vivo is often a walling off of the worm, or a marked stunting of growth, in addition to other manifestations. Studies by many investigators have been done on the effect in vitro of serum antibody on larval and adult stages of helminths. These, of necessity, have been short-term experiments capable of demonstrating only rapid responses on an organism which is surviving but not undergoing growth. With the development of suitable cultivation procedures it should eventually be possible to study the effect of serum antibody on the differentiating and maturing worm in the absence of the cellular response. Such information may aid considerably in delineating the role of these two factors in the total immune reaction.

Although such investigations in vitro would be of interest to helminthologists in particular, they may provide, in addition, a fruitful tool for the study of antibody action as a generalized phenomenon. The helminth, representing a multicellular organism of complex structure undergoing a precise pattern of growth and differentiation, offers morphological landmarks to measure the effects of antibody which are not available in unicellular organisms such as bacteria.

The complexity of the life cycles characteristic of many helminths may for a particular organism involve freeliving stages, as well as successive development of intermediate larval and adult stages in several hosts drawn from widely separated phyla. In addition, intricate patterns of migration may occur within each of these hosts continuously subjecting the organism to great environmental variations. Unraveling these beautiful patterns of differentiation has occupied the attention of parasitologists for many years, so that we now have a wealth of fascinating descriptive material on the life histories of many organisms. At this point questions arise concerning the relation of the parasite to the dynamic milieu in which it finds itself. What exactly are the biochemical and biophysical needs of a particular parasitic helminth at the various stages in its life cycle? How do they change from one larval stage to the next, and how are they differentiated from the requirements of free living relatives? These, of course, are only a few of the interesting questions for which in vitro cultivation procedures may provide another tool for investigation.

Although many life histories are known in considerable detail, others are only partially described, or are in a controversial state because of the difficulty of approaching them using in vivo methods. As an example, many problems in the life history of various species of *Strongyloides* may be considerably clarified with the development of the cycle in vitro. The dependency of a parasitic female on a male for fertilization, the differentiation between genetic and nutritional factors in the development of the homogonic and heterogonic cycles, and the possibility that the heterogonic cycle is potentially capable of continuing indefinitely are only a few of the aspects susceptible to critical study.

Finally, I should like to point out the need for in vitro methods in approaching the problems relating to the possible transmission of protozoan, viral, rickettsial, and bacterial infections by helminths. The use of organisms cultivated in vitro and demonstrably free of particular infections is almost vital, if such studies are to be conducted with the same degree of care that has characterized experimental transmissions using vectors such as insects and ticks

In this discussion I have assumed the inevitability of the development of in vitro cultivation methods for various helminths. This optimism is based on the increasing numbers of papers which have appeared in recent years, particularly in the British Isles, Japan, and this country. It is to be hoped that this cross fertilization of ideas from many sources will bring about a vigorous and healthy field of research that can contribute significantly to the general field of helminthology.

PARASITOLOGIC SURVEYS IN CALI, DEPARTAMENTO DEL VALLE, COLOMBIA

I. Incidence and Morphologic Characteristics of Strains of *Entamoeba Histolytica*

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In connection with the program of family care of the Department of Preventive Medicine and Public Health, University of Valle, under the direction of Professor Santiago Renjifo S., in ward Siloé, City of Cali, Colombia, an intensive parasitologic survey has been carried out by the writer and assistants whom he has trained. Of the 1,043 members of the 106 families in the preventive medicine program, it was planned to examine three fecal specimens from each of the persons in this family group over a 2-year period in order to obtain an accurate index of the prevalent species of parasites and the intensity of the infections in each individual and family, together with the epidemiologic, socio-anthropologic, clinical and economic implications of these parasitic infections in this community. These more general aspects of the studies will be considered in subsequent communications.

Because of the prevailing ideas that "everyone in Colombia is infected with the dysentery-producing ameba" and that "amebic dysentery seldom occurs in the native population but is common in foreigners," special attention has been focused on the incidence of *Entamoeba histolytica* in this survey in ward Siloé, which represents the poorer third of the population of Cali. For comparison, fecal specimens have been examined from San Isidro Mental Hospital, the University Hospital and from private clinic patients.

The purpose of this communication is to report on the incidence and morphologic characteristics of the different strains of *E. histolytica* which have been diagnosed from the first 1,178 fecal specimens examined.

METHODS AND TECHNICS

With few exceptions the fecal specimens were passed in the morning, brought to the parasitology laboratory of the Department of Preventive Medicine and Public Health of the University in paraffined containers and were examined that afternoon. An order form which accompanied the specimen provided the following information: name, sex, age and family; if adult, whether single or married, and name of the medical student directly in charge of the family in the preventive medicine study. Before each container was opened it was given a consecutive specimen number, and before microscopic examination was undertaken the consistency and composition of the stool were recorded, viz., formed, semi-formed, diarrheic, frankly liquid, with or without blood and mucus, and gross evidence of parasites or artefacts.

Routine microscopic study consisted of a double coverglass preparation of the unprocessed fecal film, one side comminuted in physiologic saline and the other side in iodine solution (filtered saturated solution of iodine crystals in one per cent aqueous potassium iodide), and, in addition, an iodine-stained film from the surface of a zinc sulfate centrifugal flotation concentrate (Faust *et al.*, 1938, 1939), removed with a bacteriologic loop to the microscope slide. Early in the diagnostic study it was discovered that, because of the large amount of undigested starch in the average fecal sample, which obscured small parasite objects, it was necessary to make the direct fecal films relatively thin, containing not more than one to 2 mgm. of feces. At times 2 coverglass preparations were made of the surface film from the zinc sulfate concentrate, one unstained and the other iodine-stained, to study the chromatoidals in *E. histolytica* cysts which were too few to appear in the direct films. In many of the examinations in which *E. histolytica* and other protozoa were discovered by direct or concentration technic, a portion of the original specimen was diluted in several volumes of tap water, strained through two layers of dampened

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surgical gauze, and fixed in MIF medium for subsequent examination; or, if cysts of *E. histolytica* were discovered only after zinc sulfate concentration, the diluted and strained material was centrifuged several times before MIF fixation of the sediment. This method was found to be admirably suited for supplementary study of the nuclei and chromatoidals of the cysts.

Since one of the special objects of this investigation was to obtain information on the races of *E. histolytica* in the population of this area, accurate measurement of the cysts constituted an important aspect of the problem. It was therefore necessary to determine whether shrinkage occurred by any of the technics employed other than dilution of the feces in physiologic saline. To this end several hundred cysts were measured from each of the media employed. No shrinkage developed in the MIF medium but slight size reduction was detected in iodine-stained films and in the zinc sulfate surface film unless it was examined immediately. This difficulty was overcome by the addition of 0.1 per cent glacial acetic acid to the iodine solution used in the direct and zinc sulfate films.

Because all of the essential diagnostic features of the intestinal protozoa were discernible by the technics mentioned above, singly or in combination, and because Schaudinn-fixed iron-hematoxylin films invariably cause shrinkage (Sapero *et al.*, 1942), no routine hematoxylin preparations were made.

During the first two months of the study, whenever many cysts of *E. histolytica* were found in the direct films, from 100 to 300 cysts were carefully measured to determine their size range and mean diameter. Subsequently, in every positive specimen from one to several cysts were measured. Likewise accurate sketches were routinely made of the cysts to illustrate the form of the cyst, variation in size, number and structure of the nuclei, chromatoidal bodies, as well as the amount, form and density of the glycogen material in the cysts.

RESULTS OF THE STUDY

In the first 1,178 specimens examined 301 were positive for *E. histolytica*, distributed as follows: Siloé, 228 from 828 specimens (27.1 per cent); San Isidro Mental Hospital, 58 from 146 specimens (40.0 per cent); University Hospital, 8 from 74 specimens (10.7 per cent); private patients, 6 from 100 specimens (6.0 per cent), and in fecal material at necropsy, 1 from 30 specimens (3.3 per cent). These data are summarized in Table 1. In a few instances the number of positive specimens was in excess of the number of persons diagnosed as positive, due to duplicate examinations (see Table 1).

TABLE 1

Incidence of E. histolytica *in Cali, based on the first 1,178 specimens examined*

	Ward Siloé	San Isidro Mental Hospital	University Hospital	Private patients	Autopsies
No. specimens examined	828	146	74	100	30
No. specimens positive for *E. histolytica*	228	58	8	6	1
% positive for *E. histolytica*	27.5	40.0	10.8	6.0	3.3
Persons examined	651	144	72	93	30
Persons positive	218	58	8	6	1
% persons positive	33.5	40.3	11.1	6.4	3.3

If fewer than 25 cysts or other parasite objects were found in a particular coverglass preparation, the specific number was recorded. If the number was between 26 and 50 it was recorded as one plus; if between 51 and 100, 2 plus; if between 101 and 200, 3 plus, and if in excess of 200, 4 plus. Trophozoites on which positive diagnosis of *E. histolytica* was made were found only on the direct film examination and alone in only seven of the 228 positives from Siloé, but never without accompanying cysts in the other specimens examined. This was probably due to the fact that most of the fecal specimens containing *E. histolytica* were either formed or semi-formed, whereas many of the unformed specimens were positive for trophozoites of *Trichomonas hominis*, *Chilomastix mesnili*, *Giardia lamblia* and the less common intestinal flagellates but not for amebas. In 7 other instances in Siloé and in one from the University Hospital trophozoites or precysts were found with cysts in scant numbers in the direct film, but in only two of these cases was a confirmation not made by finding cysts in the concentrate. Frequently, when as few as three to five cysts of *E. histolytica* were found in the double coverglass direct films, the concentrate yielded one to 3 plus; only in two cases, when as few as few as 2 cysts were recorded for the direct film,

TABLE 2

Relative diagnostic findings with direct and zinc sulfate centrifugal flotation films from Ward Siloé, Cali

(*Based on 228 specimens positive for* E. histolyteia)

Diagnosed by	Direct films only	$ZnSO_4$ films only	Both technics	Total diagnoses
Large race				
Trophozoites	6			
Precysts	1			
Cysts	7	96	87	
Trophozoites and cysts	2			199
Small race				
Trophozoites	1			
Cysts	3	30	13	
Trophozoites and cysts	1			48
				247
Less infection of both races in same specimen				19
Net total				228

were the concentrates negative. In contrast, more than half of the positive diagnoses were made from the zinc sulfate concentrations when the direct preparations were negative; in 55 instances (18.4 per cent of the positives) diagnosis was based on demonstration of 5 or fewer cysts in the concentrate. These findings are summarized in Table 2.

Also in Table 2 the relative prevalence of large and small races is recorded for Siloé. The large race was found four times as commonly as the small race, and the latter was diagnosed more than twice as often in the direct film, whereas the large race was discovered almost as frequently in the direct as in the concentrate preparation. This is understandable because one or two cysts of the small race could more readily be overlooked in the direct film containing *Blastocystis* and fecal artefacts, even by the magnification obtained with 10 ×, 12× and 15 × Leitz widefield oculars.

In 19 instances in the Siloé specimens both large and small races were found in the same preparation, usually only in the concentrate when a few cysts of the large race alone were discovered in the direct film; but in one case only the small race (one cyst) was seen in the direct film, and in five positive specimens the direct preparation was negative for both races.

TABLE 3

Size ranges (in microns) of strains of E. histolytica *from infected individuals in Ward Siloé, Cali*

Racial type of *E. histolytica*	No. of strains measured
Small race, typically with several rice-grain chromatoidals	19
4.14–6.21, mean 5.33	1
5.6–8.7, mean 6.8–7.7	11
6.4–11.5, mean of 8.3 to 8.9	7
Large race, typically with one to 3 or 4 sausage-shaped chromatoidals	54
8.7–16 (usually 9.6–14.4) with most means of 11.7 to 11.9	50
10–16.3 with means of 11.9 to 13.7	4

Racial Characteristics of *E. histolytica* in Infections from Siloé

Racial size. On the basis of cyst size alone it was frequently not easy to separate the strains of *E. histolytica* in the population studied. While the mean diameters of a majority of the strains of the smaller races were significantly less than a large proportion of the strains allocatable to the larger race, there was considerable overlapping between the upper range of the smaller race and the lower range of the larger race. This becomes evident by reference to Tables 3 and 4. A few strains of the smaller race had a frequency distribution from 6.4 to 11.5 μ, and one strain of the larger race had almost a similar range (7.7 to 11.5 μ). The rather remarkable range in size of cysts from the same fecal specimens is illustrated in Figs. 1, 2 and 6 (9.6 to 14.1 μ, with a mean of 11.7 μ) and Figs. 3–5 (10.6 to 16.3 μ, with a mean of 13.7 μ), both examples of the large race, and Figs. 29–31 (6.7 to 10.6 μ, with a mean of 8.4 μ) and Figs. 32–34 (6.7 to 10.6 μ, with a mean of 8.4 μ, both examples of the small race.

Morphologic characteristics. In contrast to the overlapping of the outer ranges in frequency distribution of the diameter of cysts of *E. histolytica* observed in this study, it has usually not been difficult to distinguish between the two races on the basis of combined morphologic characteristics, e.g., the nuclei, chromatoidals

TABLE 4

Racial sizes in microns of representative strains of E. histolytica *from Cali, Colombia*

Strain	Figs.	No. cysts measured	Size range	Mean
A. *Small race*				
1		10	4.14–6.21	5.33
2		10	5.7–7.6	6.8
3	58	10	5.6–7.7	6.8
4	36–39	138	5.8–8.7	6.9
5		15	6.6–8.5	7.6
6	48, 49	17	6.2–10.0	7.7
7	46	12	6.7–8.6	7.7
8	52, 54	15	6.7–8.6	7.7
9	27, 28	26	6.7–9.6	7.9
10	44, 45	17	7.2–9.2	8.3
11	32–34	107	6.7–10.6	8.4
12	29–31	104	6.7–10.6	8.4
13	35	152	6.4–10.3	8.6
14	59	10	7.7–11.5	8.9
B. *Large race*				
1		109	9.6–15.4	11.0
2		10	9.6–14.4	11.7
3	17	62	9.6–14.4	11.7
4	7, 8	121	9.6–13.4	11.7
5	1, 2, 6	101	9.6–14.4	11.7
6		116	9.6–15.4	11.8
7		10	10.6–14.4	11.9
8	11, 12	311	9.6–14.4	11.9
9		169	8.7–14.4	11.9
10		110	8.7–14.4	11.9
11		88	9.6–14.4	12.0
12		132	9.6–14.4	12.0
13	15, 16	62	9.6–14.4	12.0
14	9, 13	99	9.6–14.4	12.3
15		10	10.6–14.4	12.5
16		41	10.6–14.4	12.9
17		44	9.6–14.4	13.0
18	3–5	100	10.6–16.3	13.7

Note: For graphic representation of these size ranges and means please consult Fig. A.

and glycogen masses. (Compare Figs. 1–21 with Figs. 27–59.)

In the large race studied in this series the nuclei were always discrete organelles, in which the peripheral chromatin and the karyosome stood out distinctly in both the iodine-stained and MIF preparations; they were particularly conspicuous during the maturing of the cyst, as, for example, in Figs. 2, 4, 6, 8, and 9. Even more typical of the material studied were the number and shape of the chromatoidals, which were seen in a large portion of cysts from the same strain and from different strains. They varied in number from one to four, were sausage-shaped, commonly with a straight axis and with rounded ends; when multiple they frequently crossed one another although at times they lay parallel, and with rare exceptions were of similar size and shape (but note exceptions in Figs. 9 and 13). On iodine staining there was at times only scant evidence of glycogen inclusion in the cysts (Figs. 3, 12, 20), but more often there was a moderately to intensely dense mass, with lighter, diffuse, irregular margins (Figs. 7, 9, 17) or with relatively regular contours (Figs. 2, 5, 8, 14, 18) but less consistently spherical or ovoidal than the glycogen masses of *Iodamoeba bütschlii* (Figs. 23–26).

In the small race studied in this series the nuclei were delicate in appearance even in larger-sized cysts (Figs. 33, 34, 40, 47, 59). This characteristic was especially seen in the karyosome. Although chromatoidal bodies were less commonly detected than those in cysts of the large race, when observed these inclusions were relatively numerous (4 or more), typically the shape of long rice grains, although occasionally minute replicas of the chromatoidals of the larger race (Figs. 32 and 34 from the same specimen). Except for the delicate nuclear structure of the cysts with the smaller number of larger chromatoidals, one might be tempted to classify these cysts illustrated in Figs. 32 and 34 as belonging to the larger race. The glycogen content of the cysts of the small race, when distinguishable by intensity of iodine staining, usually occupied a relatively larger mass of the cyst than is characteristic of the large race. Moreover, it frequently honeycombed the cytoplasm, leaving only minute fingers of protoplasm, at times only in the periphery, in which the nuclei were situated. This feature was so pronounced in the small-race cysts studied in this series that at times probably more than three-fourths of the cyst volume is occupied by the glycogen inclusions.

DISCUSSION

Although there are many published reports in the medical and parasitologic literature on the incidence of *E. histolytica* in hospitals and clinics, mental institutions, prisons, military groups, children in schools and institutions, industrial communities and miscellaneous populations, rela-

tively few studies have been carried out in family groups and the communities which they comprise. Notable among these latter are the investigations of Meleney and associates (1930, 1931, 1932) and Eyles and associates (1953, 1954) in rural and urban Tennessee, and Mackie and associates (1956a, 1956b) in North Carolina. In so far as the writer has been able to discover no similar study has been carried out in Latin America. The opportunity provided for the parasitologic survey in families of ward Siloé, city of Cali, Colombia, in connection with the preventive medicine program in this community, was therefore "made to order" for conducting such a survey on the incidence of protozoan and helminthic infections in a tropical community, and for comparing the data obtained with investigations of similar nature in native populations in the more temperate climate of North America.

The epidemiologic, socio-anthropologic, economic and clinical findings of the Siloé survey are to be presented in subsequent communications after more detailed information has been obtained on these aspects of the problem. For the purpose of the present study it is sufficient to report that the families on which the investigations are being carried out comprise a poor economic segment of the population of Cali, living under very unhygienic conditions. Approximately half of these families have lived in this same environment for several years, while the other half have recently come to Cali from rural areas, attracted by the rapidly increasing industrial development of the city and promise of better wages.

Composition of the Siloé families. The size of the 106 families in the preventive medicine program varies from 4 to 20, with 6 to 12 individuals per family most common. There are typically only two generations under one roof, but it is not uncommon to have one grandparent in the home. The relatively large size of the families has created a considerable problem in obtaining fecal specimens from each member of the family, although when the family has been cooperative and the medical student assigned to study the family has been especially interested and active in the project, 90 to 100 per cent of the members of the family have thus far provided at least one fecal specimen. This high compliance score is not related to the size of the family, since several families with 10 to 20 individuals have scored as high as, or higher than other families with only 4 to 6 persons.

Incidence of E. histolytica in Siloé families. The overall incidence of *E. histolytica* in the 651 individuals examined from Nov. 1, 1956 through April 30, 1957 is 33.5 per cent (Table 1). This is somewhat lower than the incidence for the 144 persons in San Isidro Mental Hospital (40.3 per cent), all of whom have had one examination. Of the 99 Siloé families from which at least one person has submitted a fecal specimen, 52 families, consisting of 403 persons, have provided specimens from 50 or more per cent of the members of the family; 144 of these persons (35.7 per cent) were *E. histolytica* positive. Thirty-nine families, consisting of 327 persons, provided specimens from 66.6 or more per cent of the members of the family; 124 of these (37.7 per cent) were positive, and 18 families, consisting of 131 persons, had 73 (55.7 per cent) positive individuals, while 21 families, consisting of 194 persons, which submitted 110 specimens from 88 individuals, varying in number from 2 to 18 specimens per family, have been found negative on one to two stool examinations per person. These incomplete data, although not tested for significance, indicate a trend, namely that *E. histolytica* infection is much more likely to exist in certain families or at least to be more prevalent in those families than in others of this community. This is in accord with the data obtained by Meleney *et al.* (1932), Eyles *et al.* (1953) in rural Tennessee, and Mackie *et al.* (1956a, 1956b) in North Carolina.

Consistency and composition of fecal specimens from Siloé families. While a considerable number of fresh stools submitted for examination were semi-formed, and in a few instances (see Table 2) contained from one to a few trophozoites of *E. histolytica* (although in a majority of such specimens cysts were also present), no frank case of amebic dysentery was encountered. The most common food residuum in the feces was undigested starch, indicating starch intake in excess of ability to utilize this carbohydrate. Cooked yucca tubers and plantain are the cheapest foods in Cali and constitute the basic diet of the poorer people. Corn, rice and potatoes are somewhat more costly and therefore less commonly consumed by this class of the urban population of Valle, whereas beef is plentiful in the markets but is a relatively expensive supplement to the basic carbohydrates. It is suggested that the high glycogen content of the amebic cysts evacuated in the stools of this class of the population in

Cali is due to the large amount of residual starch present in the large intestine, some of which is ingested by the amebas, is converted into glycogen and thus constitutes a large portion of the food reserve of the cysts.

Size and morphologic characteristics of E. histolytica cysts studied. In presenting the results of this study it has been pointed out that there is wide variation in size of the different strains of *E. histolytica* in this community, not only in different individuals but in the same host. However, a majority of the cysts have diameters with means between 6.8–7.7 and 11.7–11.9 microns respectively, even though there is appreciable overlapping between the upper size range of the former and the lower size range of the latter group. Fig. A and Table 4 provide evidence that there are two racial groups. Morphologically these two races can be distinguished usually on the basis of their nuclei, marked differences in the number and type of chromatoidals, and the type of glycogen vacuoles.

Sapero *et al.* (1942) first provided convincing evidence from their own data and those of earlier workers that there were two distinct races of *E. histolytica*, based on the mean diameter of cysts from various strains, rather than on modes which led Dobell and Jepps (1918) to conclude that there were five races in their material. Moreover, Sapero *et al.* (1942) found that, on the average, the diameters of living cysts were one micron greater than those in fixed, hematoxylin-stained, balsam-mounted films; for the former the abscissa separating the two races was demonstrated to be 10 microns, and for the latter 9 microns. On the basis of mean diameters of unshrunken cysts the present study fully confirms this finding. (See Fig. A and Table 4.)

In 1940 the writer (Faust, 1940) provided a biref summary of his study on hematoxylin-stained films from a children's home in New Orleans which indicated that a majority of the preparations contained cysts of small size. Cysts from 9 children (size range of cysts 5.6 to 8.9 microns) contained numerous rice-grain chromatoidals. These are similar to small-race cysts from Cali. Cysts with similar size range from 16 children contained several chromatoidals which were ovoidal or bean-shaped. This type has not been encountered in Cali. A third group of cysts (size range 5.9 to 9.8 microns) from 9 children had slender rod-shaped chromatoidals with rounded ends, varying in number from one to six or seven. These likewise have not been observed in Cali. A fourth group of cysts (size range 10.1–16.9 microns) from 8 children are comparable in size and chromatoidals to the large race consistently found in Cali.

With the exception of *E. hartmanni* v. Prowazek, 1912, no previous attempt has been made to differentiate large and small races of *E. histolytica* on a morphologic basis. Brumpt (1949) stated that v. Prowazek's description was very inexact, but from original material and from the literature Brumpt (l.c. pp. 124–126) reported "*E. hartmanni*" to be cosmopolitan in distribution, "very common in the United States", having *trophozoites* usually measuring 5 to 7 microns, rarely 10 microns, and spherical or ovoidal *cysts* measuring 6 to 8 microns, rarely 10 microns (*sic*); with precysts often containing "bacillary siderophilous corpuscles, straight or curved, often acicular," which, at times in two- and four-nuceated cysts, are transformed into masses more voluminous than those of "*E. dispar*" and "*E. dysenteriae*". Illustrations of several of these cysts (Fig. 112, p. 225) conform to this description. However, two cysts designated as *E. hartmanni* (*tenuis*) by Reichenow (Nauck, 1956, Abb. 42, p. 115) *each contain* several rice-grain chromatoidals but no acicular or massed siderophilous material. The only conclusion which can be drawn from these conflicting descriptions is that "*E. hartmanni*" consists of a number of strains of the small race of *E. histolytica* and as a species name should be dropped.

E. dispar, created by Brumpt in 1925 for temperate-zone *E. histolytica*, but also described by him as cosmopolitan in distribution, is identical in size and morphologic characteristics of the cysts with the large race of *E. histolytica* consistently encountered in Cali.

An observation made several times in the present morphologic study may throw light on the variety of forms of chromatoidals seen in the cysts of *E. histolytica*, namely the longitudinal splitting of these inclusions. In one cyst (Fig. 17) this splitting apparently took place under natural conditions and others (Fig. 12) following zinc sulfate centrifugal-flotation technic. In a third specimen containing 2-plus cysts of the large race in direct unstained films, having many cysts with one or two large sausage-shaped chromatoidals, centrifugation was employed without zinc sulfate flotation to concentrate the cysts for planting in culture. Preparations of the concentrate showed

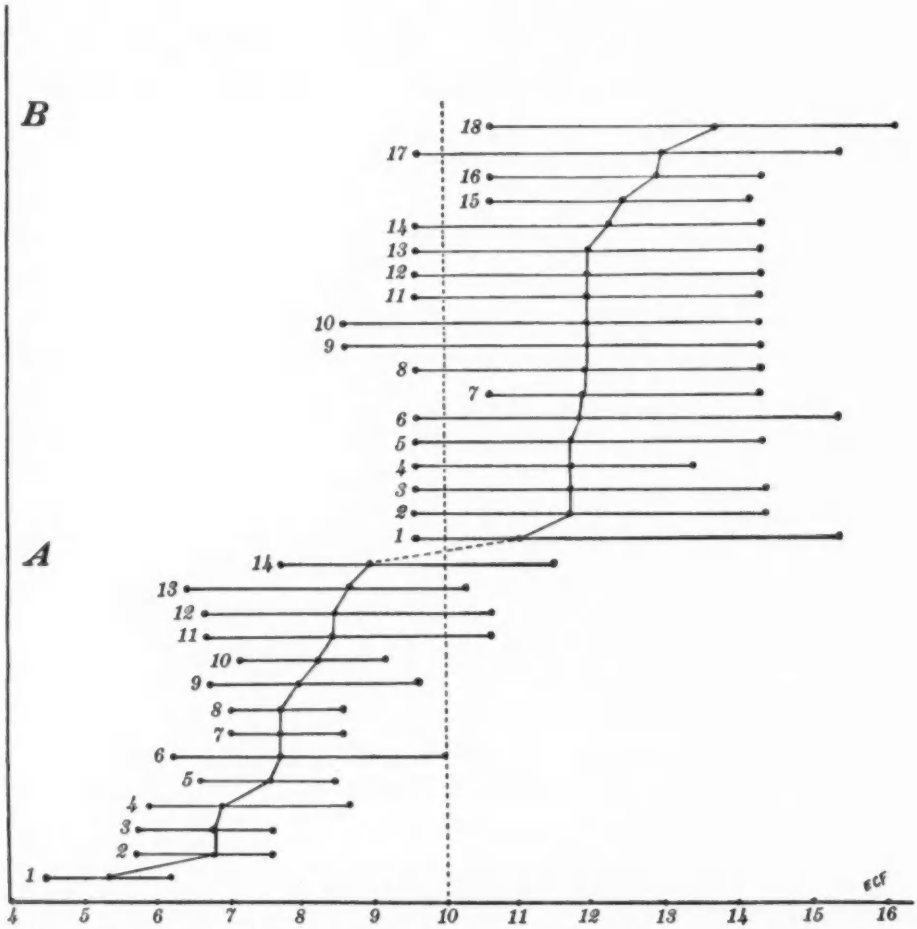

FIG. A. Graph showing the size range and means in microns of cyst diameters of *A*, 14 strains of small race and *B*, 18 strains of large race of *Entamoeba histolytica* from members of families in ward Siloé, Cali, Colombia. The sizes are indicated on the bottom line. Note the differences in mean sizes between the two races. The 10 micron abscissa separates the two races very clearly. For extremes and means of each strain, as well as the number of cysts measured, the reader is referred to Table 4.

only split chromatoidals. Other methods of processing fecal material, including fixation, may likewise possibly transform the shape and number of the chromatoidal bodies within the cysts, and this may explain the diverse forms of these inclusions described and illustrated by various workers. In the writer's experience these variations are not related to the maturity of the cysts.

The race with the larger mean diameter is the one which is regarded by many European students of amebiasis as that which is indigenous to warm climates, where it is responsible for amebic colitis and amebic liver abscess. It is the so-called "dysentery ameba." This race is considered to be uncommon in Europe, while the smaller race is fairly prevalent. On the basis of the information available thus far from the Cali study, neither race is necessarily productive of dysenteric stools in this locality. Similar information was obtained

by the writer in 1938–1939 from a study of children in orphanages in New Orleans, during an inquiry into new diagnostic procedures (Faust *et al.*, 1938, 1939). The 119 children in one institution, on a high carbohydrate, low protein diet, had an increasingly high incidence of *E. histolytica* beginning at two years of age, when they began to care for themselves (Ivanhoe, 1943). Both large and small races were found in this group but, although both races produced acute amebic colitis in susceptible laboratory animals (Tobie, 1940), there was no clinical evidence of amebiasis in any of the infected children. The situation in ward Siloé and in the San Isidro Mental Hospital in Cali appears to parallel that from the children's home in New Orleans, in which exposure occurred within the institution due to faulty personal and group hygiene.

CONCLUSIONS AND SUMMARY

A parasitologic survey was made of 990 persons in the city of Cali, Colombia, from whom 1,178 fecal specimens were collected. Stools were examined macroscopically, by direct double coverglass and by zinc sulfate flotation technics, and in many cases following fixation in MIF medium, on the day they were passed. Attention was focused on *E. histolytica*, whose prevalence and morphologic character are here reported.

The incidence of infection was 35.5 per cent in 651 inhabitants of one of the wards of the city; 40.3 per cent in 144 patients of a mental hospital; 11.1 per cent in 72 patients in the hospital of the University of Valle; 6.4 per cent in 93 private clinic patients; and 3.3 per cent of 30 fecal specimens from autopsy material.

The incidence tended to be familial since it was significantly higher in 13 selected families than in 99 others, while no positives were found in 21 families. The few trophozoites compared with the number of cysts in the stools suggests a clinically mild infection, due possibly to the large amount of undigested starch in the average stool of the group, which might tend to keep *E. histolytica* in the large intestine; however, clinical and necropsy evidence indicates that deep and extensive invasion of the tissues may occur, resulting at times in death.

Both a large and a small race of *E. histolytica* were found in the numerous strains studied. The former averaged 11.8 μ in diameter and contained 1–4 large sausage-shaped chromatoidal bodies; the latter measured most frequently between 6.8 and 7.7 μ and contained several rice-grain chromatoidals. The large race was found four times as often as the small race. The races could also be differentiated by structural characters of the nucleus as well as by cell contents, but there was no evidence that they were separate species, or that the large race was more apt to invade tissues than the small one, under the prevailing conditions.

ACKNOWLEDGMENTS

The writer is deeply indebted to all persons who have cooperated in making this survey possible—to Dr. Santiago Renjifo S. for sympathetic direction of the survey, to Srta. Inés Mora, R.N., for supervision of the collection of the fecal specimens, to Sr. Luis E. Giraldo (intern) and the medical technicians trained by the writer for diagnostic assistance, and to Srta. Lucía Marchant, the writer's secretary, who has processed the orders and diagnostic sheets and developed a system of permanent records.

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PLATE 1

FIGS. *1–21*, large race of *E. histolytica*, showing variation in size, shape and internal organization of the cysts; Fig. *22*, representative cyst of *Entamoeba coli*; Figs. *23–25*, cysts and *26*, trophozoite of *Iodamoeba bütschlii*; Figs. *27–59*, small race of *E. histolytica*, showing variation in size, shape and internal organization of the cysts. The chromatoidals, as observed in unstained and MIF-stained preparations, are outlined in relief against the cytoplasm and glycogen contents of the cysts, as revealed in iodine-stained preparations, and for convenience the nuclei are pictured with membrane and chromatin constituents darker instead of lighter than the cytoplasm.

Large race.—Figs. *1*, *2* and *6*, from a member of family 28 (with size range of cysts 9.6 to 14.4 μ and mean of 11.7 μ), show typical large race chromatoidals and intense glycogen mass consistently present in this strain. Figs. *3–5*, from a member of family 18 (with size range of cysts 10.6 to 16.3 μ and mean of 13.7 μ), depict variation in size and glycogen content in different cysts of this strain. Figs. *7* and *8*, from a member of family 62 (with size range of cysts 9.6 to 13.4 μ and mean of 11.7 μ), illustrate the consistent presence of a single large chromatoidal in this strain. Figs. *9* and *13*, from a member of family 33 (with size range of cysts 9.6 to 14.4 μ and mean of 12.3 μ), illustrate variation in size and shape of chromatoidals in the same strain. Fig. *10*, from a member of family 51 (with size range of cysts 9.6 to 15.4 μ and mean of 11.9 μ), resembles Fig. *2* except for shape and less intense glycogen mass. Figs. *11* and *12* from a member of family 29 (with size range of cysts 9.6 to 14.4 μ and mean of 11.9 μ), represent a strain with marked plasticity of form (Fig. *12* shows split paired chromatoidals following centrifugation). Fig. *14*, from a member of family 4 (with size range of cysts 8.7 to 13.4 μ and mean of 11.4 μ), is comparable to Fig. *2*. Figs. *15* and *16*, from a member of family 71 (with size range of cysts 9.6 to 14.4 μ and mean of 12.0 μ), resemble Fig. *7* with a single chromatoidal. Fig. *17*, from a member of family 13 (with size range of cysts 9.6 to 14.4 μ and mean of 11.7 μ), is relatively remarkable for its long narrow chromatoidals, consistently present in this sample in uncentrifuged material. (Compare with Fig. *12*). Figs. *18–21*, all from one specimen of feces from a member of family 42 (with size range of cysts 9.6 to 12.5 μ and mean of 11.4 μ), are characterized by their frequent irregularly ovoidal shape, variation in number of chromatoidals and in glycogen content.

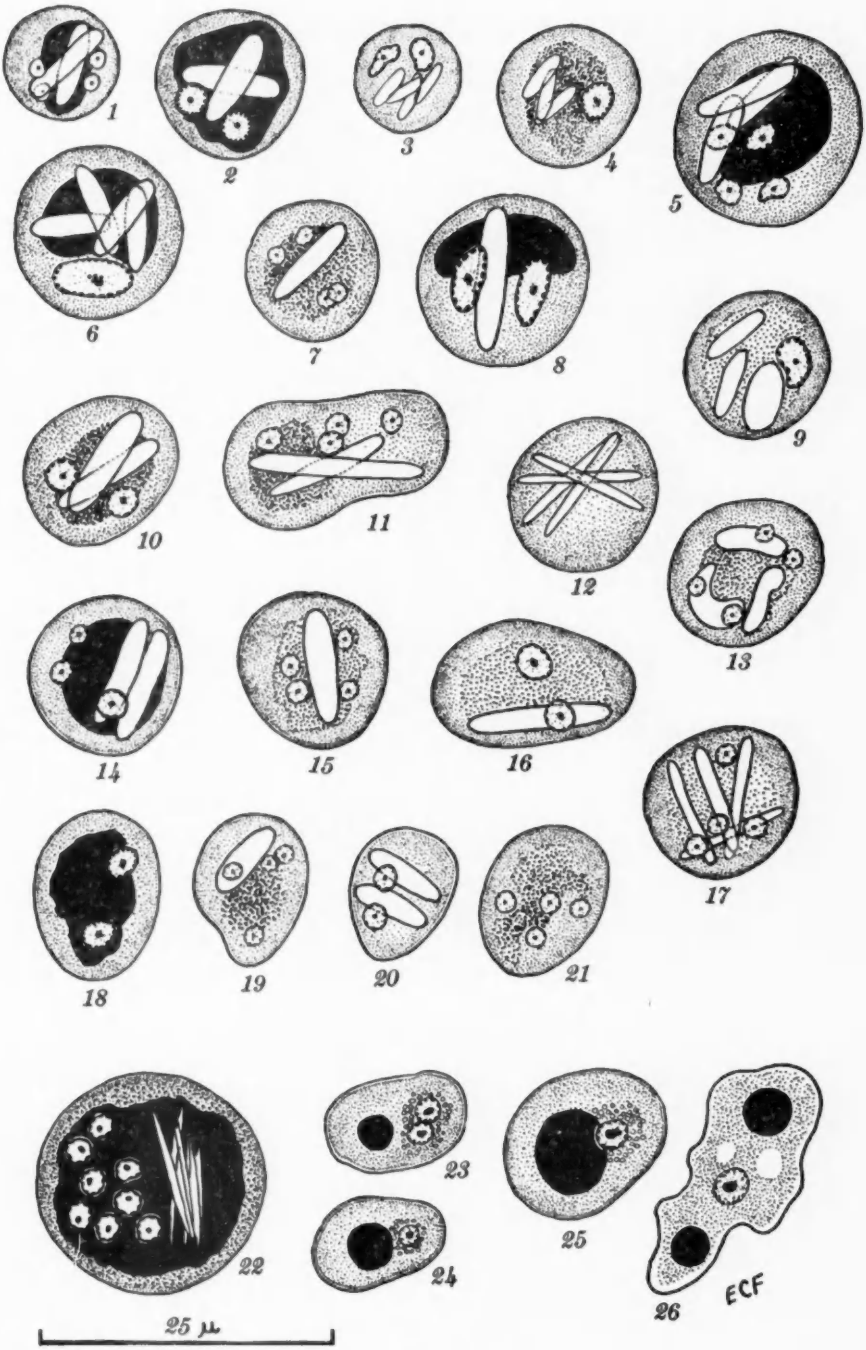

PLATE 1

PLATE 2

Small Race.—Figs. *27* and *28*, from a member of family 11 (with size range of cysts 6.7 to 9.6 μ and mean of 7.9 μ), show a dense glycogen mass characteristic of this strain and rice-grain chromatoidals in Fig. *27*. Figs. *29–31*, from a member of family 27 (with size range of cysts 6.7 to 10.6 μ and mean of 8.4 μ), represent variations in glycogen content in the small race. Figs. *32–34*, from a member of family 50 (with size range of cysts 6.7 to 10.6 μ and mean of 8.4 μ), provide evidence of variation in shape and contents of the cysts in the same sample. Fig. *35*, from a member of family 42 (with size range of cysts 6.4 to 10.3 μ and mean of 8.6 μ), is comparable to Fig. *28*. Figs. *36–39*, from a member of family 33 (with size range of cysts 5.8 to 8.7 μ and mean of 6.9 μ), are from the same fecal sample as Figs. *9* and *13* (large race). Fig. *40*, from a member of family 5 (with size range of cysts 6.6 to 8.5 μ and mean of 7.6 μ), is representative of the consistent arrangement of rice-grain chromatoidals around a dense central glycogen mass in the cysts of this specimen. Figs. *41* and *42*, from a member of family 28 (with size range of cysts 5.7 to 7.6 μ and mean of 6.8 μ), are from the same specimen as Figs. *1*, *2* and *6* (large race). Fig. *43*, from a member of family 4 (with size range of cysts 6.7 to 7.6 μ), was obtained from a different member of the same family as the large race illustrated in Fig. *14*. Figs. *44* and *45* (size range of cysts 7.2 to 9.2 μ and a mean of 8.4 μ), Figs. *50* and *51* (with size range of cysts 6.7 to 7.6 μ), Figs. *55* and *56* (with size range of cysts 6.3 to 7.7 μ), and Fig. *57* (with size range of cysts 6.8 to 8.6 μ), are from four members of family 12. Three of these persons were also infected with a large race of *E. histolytica* (not illustrated). Fig. *46*, from a member of family 64 (size range of cysts 6.7 to 8.6 μ and a mean of 7.7 μ), was also found together with a large race. Fig. *47*, from another member of the same family, had comparable small-race cysts with respect to size range and appearance. Figs. *48* and *49* are typical of a strain from a member of family 23 (with size range of cysts 6.2 to 10 μ and a mean of 7.7 μ). Figs. *52* and *54*, and Fig. *53*, from two members of family 43, represent strains with cyst size ranges from 6.7 to 8.6 μ and a mean of 7.7 μ. Fig. *58*, from a member of family 78 (with size range of cysts 5.8 to 7.7 μ) and Fig. *59*, from a member of family 99 (size range of cysts 7.7 to 11.5 μ and a mean of 8.9 μ) constitute rather uncommon form variations in the small race of *E. histolytica*.

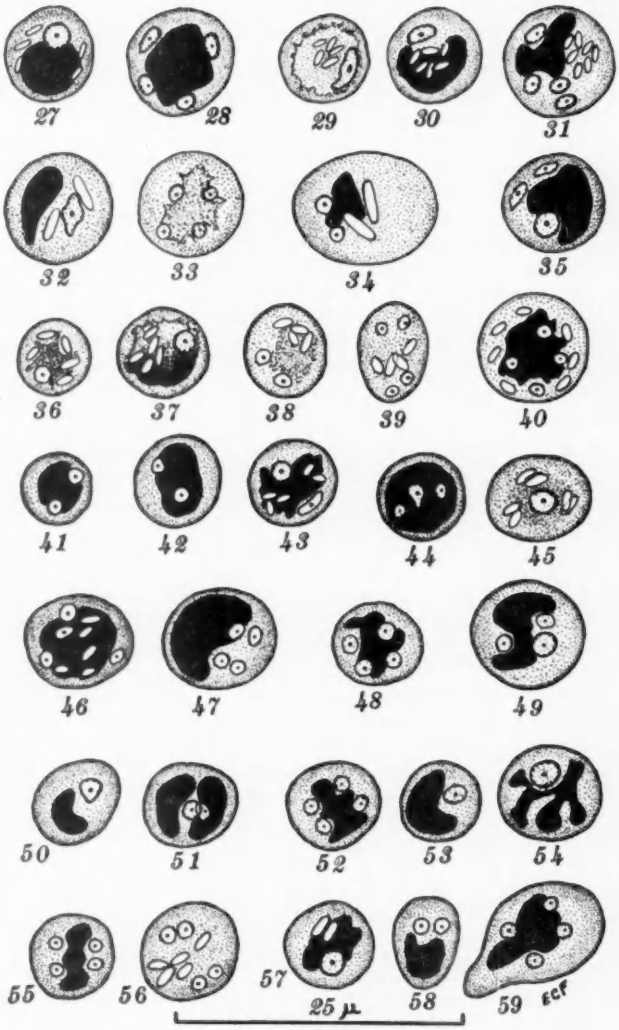
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PLATE 2

THE EFFECT OF VIABLE BACTERIA ON THE CAPACITY OF A STRAIN OF LARGE-RACE *ENTAMOEBA HISTOLYTICA* TO PRODUCE ABSCESSES IN THE HAMSTER LIVER[1]

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The pathogenicity of *Entamoeba histolytica* for liver tissue of human beings and experimental animals has been known for many years. Cleveland and Sanders (1930) reported on results of the direct inoculation of the livers of cats with *E. histolytica* which had been grown in mixed bacterial flora. They found that after a variable period of time a small percentage of the lesions produced in this way became bacteria free due, they postulated, to the bactericidal action of liver tissue. Since that time numerous references to the so-called "sterile" amebic liver abscesses in clinical material have been made (Anderson, 1953).

Although liver abscesses may be free of viable bacteria after development, thus indicating that bacteria may not be important at that time, it is not known how the lesions are initiated and workers in this field have long been interested in the possible role played by viable bacteria in the original establishment of *E. histolytica* in liver and indeed in other tissues.

Since *E. histolytica* has not as yet been successfully propagated in the absence of other viable cells, it has not been possible adequately to investigate the role of bacteria in the initiation of lesions. It seemed possible that the Shaffer-Frye (S-F) medium (Shaffer *et al.*, 1949) offered possibilities, since it contains only nonmultiplying bacteria and quite frequently no viable bacteria can be demonstrated in 48-hr. cultures. It was decided to employ the method of Reinertson and Thompson (1951) for the production of liver abscess in hamsters and to use amebas grown in the S-F medium to see if any leads could be found on the influence of viable bacteria on lesion production.

MATERIALS AND METHODS

Throughout these experiments, 48-hour cultures of the K-9 strain of *E. histolytica* in S-F medium were employed.[2] The cultures were prepared in the manner described by Shaffer *et al.* (1949) the constituents of which were multiplied five fold in order to produce larger numbers of amebas. These amebas were prepared using 12.5 ml. of a supernatant fluid from a centrifuged 24-hr. culture of an unidentified gram-negative anaerobic streptobacillus to which was added 4 ml. of a saline horse-serum penicillin base and inoculated with 2.5 ml. of a 48-hr. culture of amebas. Thus the total volume was 19 ml. placed in a 40 ml. centrifuge tube and layered with petrolatum. The contents of 4 such tubes, rated as 4 plus after 48-hours growth (Shaffer *et al.*, 1949), were spun in a refrigerated centrifuge (5°C.) at 1,000 revolutions per minute for 10 minutes. The petrolatum seals were then broken and 15 to 17 ml. of the supernatant fluid was removed and discarded. The contents remaining in the 4 tubes were pooled into a 10 ml. graduated centrifuge tube, resealed with petrolatum and spun at room temperature at 1,000 revolutions per minute for 10 minutes. The seal was broken and all but one ml. of the supernatant fluid removed. The amebas remaining in the tube formed the basis of the inoculum.

The inocula were of three different types: (1) amebas prepared as outlined above with supernatant fluid added to bring the total volume to 2 ml.; (2) amebas prepared in the same manner to which a 24-hr. culture of streptobacillus was added to bring it to the same volume; (3) amebas similarly prepared to which procaine penicillin in a concentration of 50,000 units per ml. was added to bring it to the standard volume. In all cases the inoculum was 1/10 ml. and contained an average of 14,000 to 30,000 amebas determined by direct count of a sample of inoculum in a counting chamber.

In order to determine whether or not viable

[1] This investigation was supported by a grant (E-499C4) from the Microbiological Institute of the National Institutes of Health, Public Health Service.

[2] The K-9 strain was obtained from Dr. M. M. Brooke of the Communicable Disease Center, Public Health Service. It was brought from Korea in 1951 by Dr. W. W. Frye.

TABLE 1

Results of intrahepatic inoculation of hamsters with ameba suspensions with and without demonstrable viable bacteria

	Subculture of inoculum negative for bacteria			Subculture of inoculum positive for bacteria*		
	S-F medium	S-F medium & penicillin†	Total	S-F medium	S-F medium and streptobacillus	Total
No. of animals	8	27	35	11	52	63
Positive lesions‡	0	2	2	6	28	34
Questionable lesions	0	4	4	0	7	7
Positive lesion percentage	0%	7%	6%	55%	54%	54%

* To determine whether or not the gram negative anaerobic streptobacillus was, itself, capable of producing lesions, ten animals were inoculated with $\frac{1}{10}$ ml. of a 24-hr. culture of this organism. No lesions were seen.

† Shaffer and Frye (1948) have shown that, while penicillin markedly affects the bacteria when combined with amebas in culture, the inhibition of amebic growth is minimal. Repeated cultures of this inoculum into Shaffer-Frye media were always positive for amebas. There has been no report of lesion production by penicillin itself.

‡ Of the 34 positive lesions produced in animals with inocula from which there were positive bacterial culture, 25 were sub-cultured into tubes of Shaffer-Frye media. In 20 cases (80%) amebas were observed. Positive sub-cultures for amebas were obtained from the two positive lesions where the inoculum itself did not yield bacterial growth in sub-culture.

streptobacilli capable of propagation were present, samples of all inocula were subcultured in fluid thioglycollate medium (BBL 136C), to which 1.0% dextrose had been added.

For these experiments, golden hamsters of either sex, weighing from 50 to 100 gm. were used. The animals, anesthetized with ether, were opened with a horizontal incision just under the zyphoid process and the liver exposed. Using a 1 ml. tuberculin syringe with a 26 ga. needle, the inoculum was introduced directy into the right lobe of the liver.

After inoculation of the hamsters, 1 to 2 drops of sterile thrombin topical (Parke-Davis, Inc.) was placed over the site of injection in the liver to promote hemostasis. The abdominal wound was then closed with 4 to 5 small surgical clips and the animals isolated in separate cages.

All animals were autopsied one week following inoculation. The livers were removed and examined for gross pathological lesions. Lesions 2 mm. or over in diameter were considered positive. Those lesions less than 2 mm. were considered questionable since they did not yield sufficient material for adequate examination. Wherever possible the lesions were subcultured into tubes of S-F medium incubated at 37°C. and examined at 24-hr. intervals for amebas. These cultures were considered negative if there were no amebas seen at the end of 96 hours.

RESULTS

A total of 101 hamsters were used. Thirty-six animals, one of which died due to surgical trauma, were given inocula from which bacterial cultures were negative for streptobacillus. Sixty-five animals, two of which died due to surgical trauma, were given inocula from which streptobacillus was grown. A tabulation of the lesion production in these groups is shown in Table 1.

CONCLUSIONS

Examination of the tabulated results would seem to indicate that some synergistic relationship existed between the bacteria and the K-9 strain of amebas which enhanced the ability of the latter to initiate liver abscess under the conditions of these experiments. It would seem that this relationship did not depend on the number of bacteria present. This is shown by the similar percentage of lesions produced where the inocula contained a minimal number of bacteria (from S-F medium) and where bacteria were added to the inoculum in large numbers (1 billion/ml.).

Considering the somewhat parallel situation in the intestine, McHardy *et al.* (1955) in their recommendation on therapy of amebiasis have concluded that the bacteria not only enhance the growth and multiplication of amebas, but assist in their invasiveness. One may also cite the work

of Phillips *et al.* (1955) in germ-free guinea pigs where in the absence of microbial associates, intestinal amebas appear harmless and incapable either of inducing pathologic effects or even of surviving in the intestine.

The question as to the ability of *E. histolytica* to produce this abscess alone must wait for its final answer until pure cultures of the amebas can be produced.

SUMMARY

Lesions were produced in hamster livers by direct inoculation with suspensions of *E. histolytica* (strain K-9) prepared from S-F medium. The presence of viable bacteria (streptobacillus) in the inoculum resulted in a higher per cent of positive lesions than occurred with inocula from which bacteria were not demonstrated. The addition of large numbers of bacteria (streptobacillus) to the inoculum did not increase the percentage of positive lesions.

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STUDIES ON MALARIA IN CHIMPANZEES

VI. LAVERANIA FALCIPARUM[1]

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It has been shown in this series that two human malaria parasites, *Plasmodium vivax* and *P. ovale*, will grow in the liver of chimpanzees and in the blood of splenectomized chimpanzees (Bray, 1957c). Furthermore, both parasites have been shown to persist at least for a short time in the liver of chimpanzees. The present investigation was undertaken to determine the behavior of *Laverania falciparum* under similar conditions and, in particular, to discover if *L. falciparum* persisted in the liver of chimpanzees.

MATERIALS AND METHODS

The two strains of *L. falciparum* were isolated locally. The vertebrate host used experimentally was *Pan troglodytes versus* and the invertebrate host was *Anopheles gambiae* drawn from a laboratory colony. All chimpanzees were treated with 900 mg. chloroquine base over 3 days and 105 mg. primaquine over 14 days at least one month prior to experimental use.

All blood films were stained by the Giemsa method. Liver pieces were taken from the chimpanzees by open laparotomy, fixed in Carnoy's fluid, sectioned and stained by the Giemsa-colophonium method. The infection of chimpanzees was accomplished by the intravenous inoculation of infected salivary glands of *A. gambiae*. These were suspended in a medium of 15 per cent (v/v) inactivated human serum in Locke's fluid.[2]

RESULTS

Five hundred *A. gambiae* fed on an adult human patient when 47 gametocytes of *L. falciparum*/cu.mm. of blood were present became 93 per cent infected. Sporozoites were found in the salivary glands 11 days after feeding. The salivary glands of 106 *A. gambiae* were used to infect chimpanzee No. 21 (approx. age 15 months). The inoculum showed numerous sporozoites, but at the same time a microsporidian infection of the *A. gambiae* had reduced the number of oocysts coming to maturity. Examination of 150 sections of a liver piece taken from chimpanzee No. 21 five days after infection revealed no pre-erythrocytic schizonts. Eight days after infection chimpanzee No. 21 was splenectomized and ten days after infection *L. falciparum* commenced to show in the blood. The infection remained low for 2 weeks, then rose sharply to a level of 14,600 parasites/cu. mm. All forms of erythrocytic schizogony were seen in the peripheral blood. Immature gametocytes appeared 30 days after infection and after 20 days of patency. After 22 days of patency and 8 days of heavy parasitemia the parasite density commenced to drop. Mature gametocytes were never seen despite the large numbers of immature gametocytes seen. *A. gambiae*, fed when immature gametocytes were present in the blood in large numbers, failed to become infected.

Several recrudescences of the infection occurred and at one time the infection rose to 34,000 parasites/cu. mm. Immature gametocytes also reappeared but only one mature gametocyte was seen during the recrudescences. Once more all stages of erythrocytic schizogony were seen in the peripheral blood.

Two hundred fifty *A. gambiae* fed on a 9-month-old child when 570 gametocytes of *L. falciparum*/cu. mm. of blood were present, became 95 per cent infected, with many oocysts per infected gut. Sporozoites were found in the salivary glands 10 days after feeding. The salivary glands of 115 *A. gambiae* were used to infect chimpanzee No. 18 (approx. age 30 months). Liver pieces were taken from chimpanzee No. 18 at 5, 8 and 12 days after infection. The spleen was removed 12 days after infection.

In the piece of liver taken 5 days after infection approximately 5,000 pre-erythrocytic schizonts/cu. mm. of liver were found.

In the piece taken 8 days after infection, 3 nearly mature schizonts and one degenerate

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[2] I am indebted to Dr. R. C. Muirhead-Thomson for assistance in the dissection of mosquitoes.

schizont were found in 120 sections, and in the piece of liver taken 12 days after infection no schizonts were found in some 0.5 cu. cm. of liver tissue (800 sections 6μ thick and approximately 1 sq. cm. in area). Tiny rings of *L. falciparum* appeared in the blood of chimpanzee No. 18, 6¾ days after infection and at 7 days reached a maximum of 500/cu. mm. By 7½ days the infection had dropped to 200/cu. mm. and on the 8th day after infection it had dropped to where only a few rings could be found in the thick film. The infection was still visible in the early part of the 9th day, but later on the 9th day it became invisible and remained so until the death of the animal 14 days after infection and 2 days after splenectomy. Only tiny rings were seen at any time.

L. falciparum *in the blood of the chimpanzee.* In chimpanzee No. 21 it was possible to study all erythrocytic forms except mature gametocytes. All stages were typical of *L. falciparum* as seen in the blood of man, whether in the peripheral blood or the tissue systems. They resembled exactly the forms of *L. reichenowi* seen in the chimpanzee as described by Bray (1956) except in the number of merozoites formed. In chimpanzee No. 21 the strain of *L. falciparum* employed produced in 50 schizonts an average of 27.1 merozoites (20–34) with the majority of schizonts producing 28 merozoites. The immature gametocytes seen exactly resembled those of *L. reichenowi* as described by Bray (1957 b) and did not cause the peculiar markings in the host erythrocyte described by Garnham (1933).

L. falciparum *in the liver of the chimpanzee.* The 5-day-old pre-erythrocytic schizonts were similar in all respects to the 4-day-old pre-erythrocytic schizonts of Shortt *et al.* (1951) and the smaller schizonts of Jeffery *et al.* (1952). The average diameter of the ovoid schizonts was 32.2 μ x 26.7 μ (41–28 μ) x (31–19 μ). The outline of the parasite was usually regular and without lobes.

The cytoplasm of the schizont was rarely vacuolated and the vacuoles were small and never numbered more than two. The contour of the parasite was sometimes well defined for part of the periphery although it was impossible to tell if this was due to a distinct parasite envelope.

The contour was indented by the host cell nucleus and by any fat vacuoles present in the host parenchyma cell. Three different types of nuclei could be designated, though gradations between types occurred. There were parasites with tiny dots of chromatin of about 0.5 μ in diameter usually associated with a smooth homogeneous appearance of the cytoplasm. There were parasites with round pieces of chromatin of about 1.0 μ in diameter which seemed to be an intermediate stage. There were parasites with relatively large spherical, oblong or square pieces of chromatin of 1.0–1.7 μ in length associated with a cytoplasm which tended to display aggregations of basophilic substances. All nuclei showed the same stain reaction.

The host parenchyma cell was enlarged; the host cell nucleus was pushed to one side but was not distorted or flattened as a rule and the parasite was forced to grow around it. Other than the space occupied by host-cell nuclei or vacuoles the whole host cell was occupied by the parasite. One or more of the first layer of parenchyma cells surrounding the host cell was usually slightly flattened by the pressure of the enlarged host cell, but this was minimal.

The 3 nearly mature parasites found 8 days after infection resembled the 5 and 6 day old schizonts of Shortt *et al.* (1951) and the large schizonts of Jeffery *et al.* (1952). Only one showed the lobose arms described by Shortt *et al.* (1951). The one degenerate parasite seen was 31 μ in diameter, the cytoplasm was very tenuous and the nuclei were large pale spheres (3 μ in diameter) which stained poorly.

DISCUSSION

The chimpanzee will support the growth of the tissue phase of *L. falciparum* as it does that of *P. vivax* and *P. ovale.* The splenectomized chimpanzee will support the growth of the erythrocytic phase of *L. falciparum* with the possible exception of the mature gametocytes.

The erythrocytic phase of *L. falciparum* is apparently unaltered by passage into a splenectomized chimpanzee except for the site of schizogony and gametocyte production. As with heavy infections with *L. reichenowi* the chimpanzee allows all stages of schizogony and gametogony of *L. falciparum* to occur in the peripheral blood circulation. It seems that this phenomenon is more a function of the chimpanzee than any specific characteristic inherent in the parasite, thus allowing a similar behavior of the two parasites in the same host. The phenomenon present in infections with either species of parasite removes any chance of differentiating be-

tween the two species on this basis. The other possible morphological basis for differentiation between *L. falciparum* and *L. reichenowi* is the number of nuclei produced. One strain of *L. reichenowi* is known to have produced an average of 12 merozoites in the blood of chimpanzees (Bray 1956), and the strain of *L. falciparum* used here produced an average of 28 merozoites. This, however, can only be claimed as a strain difference, as various numbers of merozoites (6–32) have been reported in *L. falciparum* infections (see Bray 1956).

The inability on the part of the strain of *L. falciparum* in chimpanzee No. 21 to produce mature gametocytes though immature gametocytes were present resembles the similar situation occurring with *L. reichenowi* (Bray, (1957 b)) Thus, the phenomenon appears to be bound up with the chimpanzee, particularly when heavy infections are induced by splenectomy.

No gametocytes were seen at any time in howler monkeys infected with *L. falciparum* by Taliaferro and Taliaferro (1934) and Taliaferro and Cannon (1934). In this case however blood inoculation was used and immature gametocytes were not seen; thus this phenomenon appears to be a case of inability to produce gametocytes rather than the inability of gametocytes to become fully mature.

Chimpanzee No. 18 displayed a strong resistance to the growth of the erythrocytic phase of *L. falciparum*. Obviously when the spleen is present the chimpanzee can eradicate the blood forms of *L. falciparum* swiftly and efficiently. The merozoites from the tissue phase are able to enter chimpanzee erythrocytes and some growth occurs, but they are removed rapidly and there is no evidence that even a single generation was able to reproduce, as the forms seen on the 9th day were the tiny rings typical of those produced from the pre-erythrocytic generation and schizonts were still bursting in the liver on the 8th day. This is contrary to the report of Lefrou and Martignoles (1954) who claim to have infected 2 chimpanzees with *L. falciparum* by blood inoculation, but is supported by the work of Mesnil and Roubaud (1920), Blacklock and Adler (1924), Rodhain (1939) and Rodhain and Muyelle (1938), all of whom failed to infect chimpanzees with *L. falciparum*.

The pre-erythrocytic schizonts found in the liver of chimpanzee No. 18 accord well with previous descriptions of these stages by Shortt *et al.* (1951) and Jeffery *et al.* (1952). The period of time occupied by the development of the pre-erythrocytic generation is about 6¾ days in the chimpanzee rather than the 5½ to 6 days recorded for *L. falciparum* in man by Fairley (1947) and Shortt *et al.* (1951).

For 20 years or more it has been known that *L. falciparum* differs from *P. vivax*, and probably *P. malariae* and *P. ovale* in certain characters which argued a probable major difference in the life cycle. The 3 main characters in which this difference could be discerned are:

1. The pattern of parasitemia, over a period of time, of *L. falciparum*, which differs radically from that of *P. vivax* (James *et al.*, 1936);

2. The extension of this to show that in *L. falciparum* infections no true relapse occurs. That is to say that, although recrudescences of an existing parasitemia occur, there is no period of proven aparasitemia between recrudescences such as occurs in *P. vivax* infections between relapses (Fairley 1947).

3. The chemotherapy of *L. falciparum* infections shows that schizonticides such as quinine and Atabrine and partially prophylactic drugs like proguanil may be radically curative, which is not the case with *P. vivax* infections (Ciuca *et al.*, 1937; Fairley, 1946).

These facts led various observers (Davey, 1946; Fairley, 1947) to postulate a lack of continuous tissue development in the case of *L. falciparum*, though Huff (1947) warned that it was possible that the later stages might exist but be more susceptible to chemotherapy. The discovery of the exo-erythrocytic stages of mammalian malaria parasites by Professors Shortt and Garnham led them to postulate a continuous exo-erythrocytic cycle in the case of *P. vivax*, *P. cynomolgi* and *P. malariae* but the occurrence of a tissue phase of only one pre-erythrocytic generation in the case of *L. falciparum* (Shortt and Garnham, 1948; Garnham, 1951; Shortt *et al.*, 1951). Finally, Garnham (1953) became sufficiently convinced of the justice of this claim to urge the use of the genus *Laverania* to include the malignant tertian malaria parasites of man and higher apes.

The experiment communicated here was designed to disprove this claim if possible, since experiments to prove it are beyond the present techniques. The absence of exo-erythrocytic schizonts of *L. falciparum* in 0.5 cu. cm. of liver taken 12 days after infection, when compared

with the finding of appreciable numbers of exo-erythrocytic schizonts of *P. vivax* 15 days after infection (Bray, 1957a) and *P. ovale* 18 and 39 days after infection (Bray, 1957c) under similar experimental conditions is consistent with the postulation of a single generation in the tissue phase of *L. falciparum*. On the other hand, it cannot be said to prove the postulation unless the whole liver were searched in several similar experiments.

The inferences to be drawn from relapse and recrudescence patterns, chemotherapeutic response and the lack of contrary experimental proof outlined here has convinced me that the postulation is true. Furthermore, if the postulation is accepted as correct, the case both phylogenetically and morphologically is overwhelmingly in favor of the separation of the malignant tertian malaria parasites into a genus of their own.

The genus *Laverania* exists for this purpose and the use of the genus by those who desire to distinguish the malignant tertian malaria parasites generically is allowed by the ruling on these parasites made by the International Committee on Zoological Nomenclature (Hemming, 1954). Opinion No. 283 leaves this genus open for use by those who considered that the crescentic gametocyte of the malignant tertian malaria parasites is a character conferring generic status. This is obviously a worthless character in this context, as it is possessed by many malaria parasites of birds and reptiles which bear no intrageneric relation to the malignant tertian malaria parasites of man and apes.

The genus *Laverania* is therefore redefined as containing obligate protozoan parasites which reproduce sexually and by sporogony in an insect host and asexually by schizogony in two phases in a vertebrate host, one in red blood cells producing pigment and the other for one generation only in liver cells. The genus contains two species, the type species *L. falciparum* of man and *L. reichenowi* of higher apes. The use of the genus is not obligatory.

SUMMARY

Laverania falciparum will grow in the liver of a chimpanzee and in the blood of a splenectomized chimpanzee. The tissue phase in the chimpanzee accords with previous descriptions in man, but the time of pre-erythrocytic development is a little longer in the chimpanzee. In the peripheral blood of a splenectomized chimpanzee *L. falciparum* was seen at all stages except the mature gametocytes. No second generation of exo-erythrocytic schizogony or persisting exo-erythrocytic schizonts could be found in the liver 12 days after infection. On the basis of this and other evidence the choice is made to assign the parasite to the redefined genus *Laverania*.

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YELLOW FEVER IN THE LOWER MOTAGUA VALLEY, GUATEMALA

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On January 5, 1956, news of scattered monkey mortality occurring in the lower reaches of the Motagua Valley was received in the Pan American Sanitary Bureau's Zone III Office in Guatemala City. The territory involved, according to rumors, was along the San Francisco del Mar river, a deep and narrow channel which drains the marshy forest area situated east of Puerto Barrios into the Caribbean. The first observations had been made, as usual, by hunters whose hounds were distracted from their duties by the moribund monkeys or by their decaying carcasses; later, squatters and farmers found cadavers along the trails and around their corn fields. The preliminary investigation, backtracking in the general direction from which the phenomenon had apparently originated, disclosed that the first monkey deaths were observed during the last week of October in the vicinity of Jimerito (see map), close to the Honduranean border; from there it had progressed toward the San Francisco del Mar river.

This occurrence opened a new chapter in the six year old history of sylvan yellow fever in Middle America (Trapido and Galindo, 1956). The wave of virus which had raged through Panama, Costa Rica, Nicaragua and eastern Honduras from 1950 to 1954 had apparently stalled in August of that last year east of the Ulua Valley. Now it was resuming its course, after having silently conquered an ecological hiatus where conditions seemed most unsuitable for its propagation and after having weathered one of the most prolonged and severe dry seasons in the history of the region (October 1954–July 1955).

At the very time when monkeys were dying in Jimerito, an immunity survey was being conducted in the lower Motagua Valley, 20 miles upstream in Virginia, Morales and Tenedores. Out of 27 monkey sera obtained, one was a clear cut positive for yellow fever and four others were inconclusive. Further knowledge (Boshell and Groot, 1957) on heterologous and related antigens present in the region allows us to discard this result as evidence of previous contact of the monkey population with the yellow fever virus. The reports of the neutralization tests, incidentally, reached the Zone Office the same day as the news of monkey mortality on the San Francisco del Mar.

Description of the region. We shall consider as Lower Motagua that part of the valley which is included between the mouth and a point 10 kilometers S.W. of Los Amates, 50 miles up river. It is enclosed to the northwest by the Montaña del Mico (with Cerro Tipón and Cerro San Gil), and to the southeast by part of the Merendón range (with Sierras del Gallinero, Espíritu Santo and Omoa). From the mouth to Los Amates, the valley funnels down from a width of thirty miles to approximately seven miles. Above Los Amates, a rain shadow, caused by the heightening of the Minas range (southwest of the Montaña del Mico and in continuity with it) which shelters the valley against the prevalent northeasters, determines an abrupt change in the conditions of the region; the rain forest gives place, almost without transition, to a semi-desertic vegetation known as the "Zacapa" type.

To the southeast, the high Merendón wall separates the Motagua Valley from the Highlands of Santa Bárbara (Honduras). Further north and east, the Omoa range, of lesser altitude especially toward its end, affords communication and forest continuity with the lower part of the Ulua Valley. To the northwest, the rain forest system of the Motagua finds no interval as it spreads over the piedmont, the slopes and the ridge of the Montaña del Mico into the Lake Izabal basin. The consequences of this from an epidemiological standpoint are considerable. The forest which covers the south shores of the lake is in direct continuity with that to the north. The Izabal region can therefore be considered as a gateway to the Petén and Southern Mexico.

The bottom of the Motagua Valley has been for many years under cultivation by the United Fruit Company. The center of operations, originally in Quiriguá and Virginia, has been displaced in the last few years down the valley to Morales and Bananera. Surrounding the UFCO

TABLE 1

Certain climatic data by month at Quiriguá

	Temperature in degrees farenheit				Rainfall, Inches
	Maximum	Mean of maximum	Minimum	Mean of minimum	
Dec. 55	88	80	58	70	8.00
Jan. 56	90	79	52	63	2.50
Feb.	89	86	64	68	.55
Mar.	94	86	58	65	2.21
Apr.	101	91	65	70	.60
May	95	87	65	71	8.75
Jun.	95	87	70	72	14.45
Jul.	92	89	70	75	10.08
Aug.	92	84	70	72	10.55
Sept.	96	89	70	72	10.10
Oct.	98	85	64	72	7.22
Nov.	88	80	62	68	9.75
Year average		85		70	
Year total					84.76

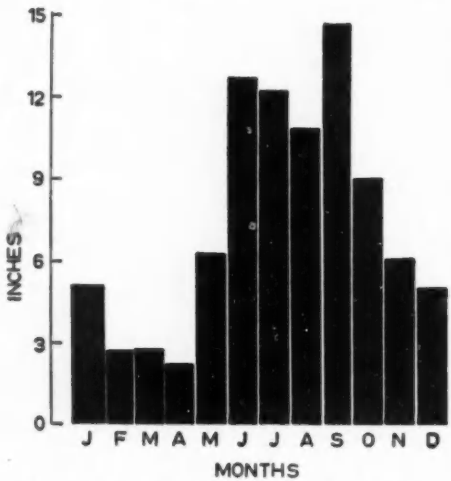

FIG. 1. Average of 18 years' rainfall at Quirigua, 1938–1955, in inches by month.

holdings there is a belt of small settlements, mostly located on the wooded foothills and on abandoned UFCO lands. Representative of these are small communities like Jimerito, Entre Ríos, Quebradas, and Virginia.

These settlements have resulted in a devastation of the primitive forest that formally covered the area. Large areas are occupied by banana or abacá plantations; others by the shrubs and thickets which are the inevitable consequence of the native system of agriculture in which a corn crop is followed by four years of free growth. The forest has therefore been reduced to patches, irregularly scattered along the floor of the valley and over the foothills. The mountain slopes are slowly being invaded but still show relatively large areas of solid forest.

The valley bottom is marshy, especially during the rainy season (or rather the season of more abundant rains) when the streams overflow the lowlands. This is more manifest in the lower part of the valley, downstream from Tenedores and Entre Ríos, in spite of vast and costly drainage works engineered by the UFCO.

Rainfall and temperature. Table 1 and Figure 1 give an idea of the rainfall in the Motagua. Table 1, which shows the temperature and rain fluctuation during 1956 may be considered as representative of the conditions in the valley; it can be seen that the increase in rainfall occurs in June and that heavy rains persist until September–October. A relative recession in the so-called "canicula" of August is inconstant. The only period which can be qualified as dry occurs in February, March and April when the amount of precipitation often falls below the monthly minimum of 6 cm. generally associated with tropical "rain forest."

As regards temperature, the chief feature during this year of observation consists of a remarkable drop of the minimum in December and January as shown in Table 1. As will be seen below, it is believed that such falls in temperature may constitute a factor of inhibition in the activity of forest mosquitoes.

Primate fauna. Only two species of wild primates are to be found in the Motagua: the spider monkey, *Ateles geoffroyi vellerosus* and the howler monkey, *Alouatta palliata*. The *Cebus* genus has its northern and western limit in northwestern Honduras and does not exist in Guatemala. This has a certain importance. The *Cebus* survives the yellow fever virus infection and therefore plays an important rule in the rebuilding of a susceptible primate population; this is in contrast with the genera *Ateles* and *Aloutta*, which show a high percentage of mortality when exposed to the virus in favorable conditions for transmission.

In the areas of old settlement the *Ateles* are

rare and often extinct; this is due to the fact that their meat is highly appreciated by the natives. The *Alouatta*, to the contrary, survives human settlement, protected as he is by his foul taste. The remnants of forest in the Motagua Valley's bottomlands are exclusively inhabited by *Alouatta*. The *Ateles* are to be found only on the mountain slopes, on the fringe of the settled areas, and deep into the forest. Neither of the two genera exhibits the extraordinary density which was one of the most characteristic aspects of the Panamanian, Costa Rican and Nicaraguan forests before the yellow fever wave.

Mosquito fauna. Due to the character and object of this study, we shall limit ourselves to the known or suspected vectors of yellow fever which can be found in the Motagua Valley. Of these, we have two species of the *Haemagogus* genus, *Haemagogus mesodentatus* and *Haemagogus equinus*, and one of the Sabethini tribe, *Sabethes chloropterus*.

The distribution of *H. mesodentatus* appears to be more specialized than that of *H. equinus*. The former is always found in greater abundance on the mountain slopes; only exceptionally has it been captured in significant numbers on the floor of the valley. This is in contrast with *H. equinus*, whose distribution includes the lowlands as well as higher levels. *S. chloropterus* is also an ever-present species, universal in its distribution and remarkably resistant to adverse climatic conditions.

All three species can definitely be termed canopy-dwellers. Only in exceptional circumstances are significant numbers captured on the ground, usually where the canopy is broken and sunlight filters through. Certain topographic accidents may occasionally favor ground captures, as where the canopy comes in contact with a large rocky formation or a perpendicular slope. On one occasion a relatively large number of *Haemagogus* were captured at ground level under a shelter where the personnel were weathering a heavy cloudburst. Discounting these exceptions, the overwhelming evidence is to the effect that the contrast between the canopy and the ground is more pronounced with these species than with *H. spegazzinii*. This may account to a certain degree for the scarcity of human cases observed so far, after crossing the geographic limit of *H. spegazzinii*.

Area of operation. The area chosen for the operations was the forested south-eastern slope of the Montaña del Mico. Captures were concentrated in two localities, one called Río Blanco, 35 Km. west of the town of Morales and the other, in a second phase of the investigation, at Cerro Tipón located 30 Km. further up the valley along the Carretera del Atlántico, on the same slope. The captures were made at altitudes varying between 150 and 700 meters.

The reason for selecting these two places was the finding, or the report of recent findings, of monkey cadavers. In Río Blanco, where the operations were started on March 23, two dead howlers had been discovered by wood cutters twelve days before. Several weeks later other reports came of monkeys having died at a higher altitude, and the capture site was moved accordingly. On Cerro Tipón a moribund howler was found on May 12 during an investigation undertaken on the basis of rumors of monkey deaths. The liver proved later to be positive for yellow fever and virus was recovered from a glycerinated liver specimen. Captures were started immediately in that area and the activities of the personnel were, from then on, distributed between the two localities.

The Motagua Valley afforded exceptionally favorable conditions for an investigation. The generous hospitality of the United Fruit Company, its permanent supply of ice, its transportation facilities, and the nightly train to Guatemala which made possible the shipment of the thermos jugs, the day after capture, by the noon plane to Panama were decisive factors in the success of the operations. To this can be added the fact that the zone of operation was parallel to a fairly good road, the Carretera del Atlántico, which is still under construction and which became impassable only in extreme conditions of bad weather.

Program and methods of investigation. The observation of mosquito behavior in the Motagua was secondary to the real scope of the work which consisted in attempts at virus isolation from suspected vectors and the gathering of evidence as to the role these played in transmission of the disease. It was decided to collect mosquitoes in selected localities and ship them to the Gorgas Memorial Laboratory, Panama, where the material was to be ground up and inoculated into laboratory animals.

Starting on March 23, daily mosquito captures were undertaken. Local laborers were trained to capture mosquitoes in the canopy without dam-

aging the specimens or allowing them to engorge, and after a few initial difficulties they became quite efficient. The captures started usually at 9:00 or 9:30 A.M. and lasted for four hours. Captured mosquitoes were kept in individual 1.5 × 4 cm. vials until the captures ended, when they were transferred to a common, 8 oz. fruit jar previously lined with a thin coating of plaster of Paris. The jar was chilled with ice in a thermos before the transfer, and after receiving the harvest, was enclosed in a waterproof plastic bag before being returned to the ice. The shipment of the thermos was made by the night train from Puerto Barrios to Guatemala, which stops at Bananera at 7 P.M., and reaches the capital by 7 o'clock the following morning. This gave time to renew the ice at the Zone Office and to catch the noon plane to Panama. Captures had to be interrupted on Fridays and Saturdays due to document-processing difficulties on the week-end. This tempo was kept up until July, when economic considerations and excessive pressure on laboratory facilities imposed a reduction of the number of captures.

Virus isolation from mosquito batches classified by species was done at the Gorgas Memorial Laboratory in Panama. This phase of the work has been described in a separate paper (Rodaniche and Galindo, 1956).

Synoptic table of events. In Tables 2 and 3, the outstanding events of this study are listed in chronological order starting in January.

TABLE 2

Observations on mortality of primates in Motagua Valley

Date 1956	Place	Type of information
Jan. 5	Lower course of San Francisco del Mar River	Reports from civilian authorities, alerted by settlers.
Jan. 6–10	San Francisco del Mar River banks and Jimerito region	Finding of 2 cadavers of *Alouatta palliata*. One of them yielded a liver specimen diagnosed as positive for Y.F.
Feb. 3	Vicinity of Virginia, Montaña del Mico	Reports from authorities.
Feb. 10	Montaña del Mico, vicinity of Puerto Barrios, Km. 223 of the road to Guatemala	Finding of 4 cadavers of *Alouatta*; 1 liver specimen diagnosed as positive for Y.F.
Feb. 11	Ibid.	Finding of 4 decomposed cadavers of *Alouatta*.
Feb. 25	Montaña del Mico, vicinity of Virginia	Finding of 1 cadaver of *Alouatta*; liver diagnosed as positive for Y.F.
Feb. 27	Cerro San Gil, Municipio Barrios	Reports of monkey mortality from hunters and woodsmen.
Mar. 3–9	Foothills of Merendón range, Las Quebradas	Several dead *Alouatta* in advanced state of decomposition found by scout.
Mar. 12	Valley floor, 5 km. southeast of town of Virginia	Finding of a dead *Alouatta*.
Mar. 20	Rio Blanco, vicinity of Km. 223 of road Guatemala-Barrios	Scattered remains of 3 *Alouatta* found.
Apr. 9	Rio Blanco, 2 miles east and 600 feet higher than locality above	2 cadavers of *Ateles vellerosus* found.
Apr. 13	La Cumbre, ridge of Montaña del Mico at the level of Km. 223, 300 feet higher than locality above	2 dead *Ateles* found in various stages of decomposition.
May 6	Same area	2 dead *Ateles*.
May 12	Cerro Tipón, altitude 350 m.	A moribund *Alouatta* found; liver proved to have specific lesions of Y.F.; virus isolated from glycerinated specimen.
May 13	Same area as May 6	2 dead *Ateles*.
June 10	Cerro Tipón	Skulls and bones of 2 *Ateles* found.
July approx. middle of month	Morjá Valley, Guatemala-Honduras border	Retrospective reports of *Ateles* mortality gathered in September.

TABLE 3

Virus isolations from pools of mosquitoes captured in Motagua Valley

Date of pool's capture	Number of mosquitoes in pool	Species	Place	Virus isolated
March 21–25	47	*Haemagogus mesodentatus*	Rio Blanco	Yellow fever
March 21–Apr. 4	59	*Haemagogus equinus*	Rio Blanco	Yellow fever
March 26–Apr. 4	65	*Haemagogus mesodentatus*	Rio Blanco	Yellow fever
April 5	67	*Haemagogus mesodentatus*	Rio Blanco	Yellow fever
April 9	115	*Haemagogus mesodentatus*	Rio Blanco	Yellow fever
April 10	112	*Haemagogus mesodentatus*	Rio Blanco	Yellow fever
April 9–10	48	*Sabethes chloropterus*	Rio Blanco	Ilhéus*
April 11–12	112	*Haemagogus mesodentatus*	Rio Blanco	Yellow fever
April 15	56	*Haemagogus mesodentatus*	Rio Blanco	Yellow fever
April 24–25	50	*Haemagogus mesodentatus*	Rio Blanco	Yellow fever
May 6	89	*Haemagogus mesodentatus*	Rio Blanco	Yellow fever
May 6	78	*Sabethes chloropterus*	Rio Blanco	Yellow fever
May 7	44	*Haemagogus mesodentatus*	Rio Blanco	Yellow fever
May 7	122	*Sabethes chloropterus*	Rio Blanco	Yellow fever
May 6–13	31	*Haemagogus equinus*	Rio Blanco	Yellow fever
May 8–13	54	*Haemagogus mesodentatus*	Rio Blanco	Yellow fever
May 9–13	122	*Sabethes chloropterus*	Rio Blanco	Yellow fever
May 14–22	47	*Sabethes chloropterus*	Cerro Tipón	Yellow fever
June 18	39	*Haemagogus equinus*	Cerro Tipón	unidentified, not yellow fever
June 20	174	*Haemagogus mesodentatus*	Cerro Tipón	Yellow fever
June 20	139	*Haemagogus equinus*	Cerro Tipón	Yellow fever
July 20	35	*Haemagogus mesodentatus*	Cerro Tipón	Yellow fever
July 26–27	148	*Haemagogus mesodentatus*	Cerro Tipón	unidentified, not yellow fever
July 29	86	*Haemagogus mesodentatus*	Cerro Tipón	Yellow fever

* (Rodaniche and Galindo, 1957).

REMARKS AND DISCUSSION

Penetration of the yellow fever wave into Guatemala. It seems a well established fact that the pathway of penetration was through the Jimerito region. It was the expected route, and the Cuyamel area on the other side of the border in Honduras had been visited several times during 1955; no evidence whatever of virus activity had been found on those occasions, nor had any indicative report been obtained from the alerted authorities and natives.

The last monkey deaths, in August 1954, had occurred in the coastal plain of La Masica (Honduras). Between that point and the Cuyamel-Jimerito sector lies the Ulua Valley. The utter and widespread destruction of forest in that part of the country had led the observers to believe that it might constitute a temporary and even a permanent obstacle to the march of the wave. Along one path only did it seem possible for the monkey-mosquito cycle to survive and progress: the marshy forest east of Puerto Cortés linked with the Lancetilla forest, which in turn is the end-point of the jungle which covers the coastal plains and foothills of northern Honduras (Nombre de Dios Range) and includes la Masica. From the forest of Cortés, the logical way of propagation was across the Ulua and the Chamelecón river along which some remnants of forest populated by *Alouatta* are still to be found. The western affluents of the lower Chamelecón have their headwaters in the wooded, eastern slope of the low Omoa Range, in close contact with the headwaters of several streams which flow into the Motagua, among them the Jimerito river.

Such a possibility, although acknowledged at the time (Boshell, 1956) did not seem to offer much probability. The reason for this was that the only mosquitoes to be found in the area were *H. equinus* and *S. chloropterus* whose transmitting ability was questioned in those days, and whose density, furthermore, had been reduced to

extremely low figures by the prolonged dry season. Even after the workers of the Gorgas Memorial Laboratory, confirming previous data from Brazil and Colombia (Laemmert *et al.*, 1946, and Boshell, 1944, unpublished reports), obtained indubitable transmission with the local strains under experimental conditions (Galindo *et al.*, 1956), those species were considered as vectors of low efficiency.

An immunity survey in September 1956 among the monkey population inhabiting the margins of the Chamelecón river gave an immunity rate slightly above 10 per cent. This is not as high as may be expected in regions where *H. spegazzinii* or *H. mesodentatus* are present; we must make allowances for the low intensity which seems to characterize the transmission by *H. equinus* and *S. chloropterus*. This sporadic mechanism permits the survival of a large number of monkeys, and just as it causes a mild mortality, it may be expected to leave a reduced immunity rate in its wake. In the present case, the phenomenon was accentuated by the exceptionally severe conditions of drought which prevailed during the first half of 1955. All these factors contributed to the failure to detect the process for several months.

It is not within the range of this work to discuss the mechanisms which favored the survival and the progress of the virus through this critical area. It need only be said at this stage that prior to the San Francisco del Mar developments, at the time of apparent epizootic silence, the theoretical possibility of a creeping, undetectable process was pointed out in various reports and articles (Boshell, 1955; 1956). This was based on previous experience gathered in other areas and always with special reference to certain types of mosquito vectors. The fact that the possibility of the virus surviving and progressing was foretold in the absence of any observed manifestation and on the basis of characters of the known local vectors, cannot be considered as definite proof of a mechanism, but is a strong suggestion that at least some of the factors involved are within our grasp.

Fluctuations of vector species. Figure 2 shows weekly averages of different quantities of *Haemagogus* per man-hour captured during this study. It must be emphasized that the figures on which it is based do not represent systematic captures at fixed stations and at exactly the same hours. The type of investigation which was being conducted necessitated the frequent displacement of capture stations to sites which afforded the greatest probabilities of success.

The density graph is based on the mean of the numbers of mosquitoes captured by five to seven mosquito catchers in several hours. These men were dispersed over an area roughly estimated at 2,500 square meters, on different grades of a slope. They represent therefore the mosquito density over a certain area but do not express what might eventually happen at any particular point. To illustrate this, we are listing the figures of one day's capture at Cerro Tipón, taken at random.

Catcher	*Haemagogus* spp.	*Sabethini*
No. 1	23	28
2	3	1
3	4	6
4	25	22
5	9	2
6	5	3
7	4	2

The mean per man-hour is 2.6 *Haemagogus*, but this is far from expressing the kind of attack a monkey passing through the area might be subject to. With these reservations it is felt that the fluctuation curve in Figure 2 represents a relative index of the mosquito population density throughout the year and that it may be considered as roughly representative of what happens in reality.

These data from Guatemala are in general agreement with the findings in Nicaragua and Panama although different species are concerned and different methods were practiced (Galindo *et. al.*, 1956; Trapido and Galindo, 1957; Galindo and Trapido, 1957).

One of the striking facts here is the lack of that correlation with the curve of rainfall which observers of tropical conditions are accustomed to find in countries situated south of the 14th parallel. If, on the one hand, it is evident that the hatching of mosquitoes that occurs in May depends on the flooding of the tree holes following the onset of rains after the February–April dry season, on the other we see that in August, September and October there is a substantial decrease in the mosquito population at a time when the amount of rainfall has not diminished in any decisive manner. These reduced mosquito numbers persist and even fall further throughout the

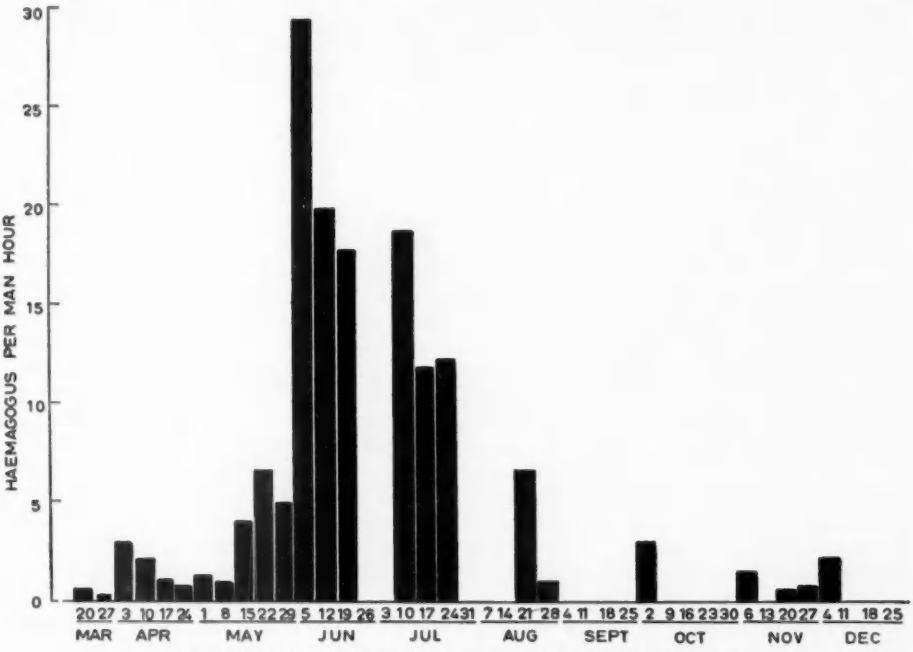

FIG. 2. Weekly averages of *Haemagogus* caught per man-hour

period corresponding to the northern winter, notwithstanding the rainfall.

In the same way, it would appear that the decrease in volume of captures which starts in August–September is independent of the temperature factor. However, there are reasons to believe that certain thermal variations may influence the activity of mosquitoes, stimulating or inhibiting it. In the beginning of April, for instance, an unexpected increase in mosquito numbers was observed following a slump which had been ascribed to the increasingly severe drought conditions. The volume of captures jumped from 0.10 to 2.7 per man hour. No significant rainfall such as would be necessary to flood breeding holes had occurred. No *H. equinus* males, which usually appear before and during the female hatching and which are the only male *Haemagogus* seen in nature at these latitudes, were observed. Moreover, yellow fever virus was recovered from several batches of those mosquitoes, showing that they were probably not the product of a recent hatching. The only factor which had varied at the time was the minimum temperature, which had on those days shown a definite upward trend. The reaction to cold of *Haemagogus* mosquitoes, which exhibit a numbed appearance a few minutes after being introduced into a barely chilled jar, seems to subtantiate the hypothesis of a temperature threshold under which the activity of adult mosquitoes is inhibited. It would thus appear that the spring hatching of the mosquito population depends on the onset of rainfall, but that its autumnal decline is independent of it. An increase in temperature may be an influencing factor in stimulating the activity of numbed mosquitoes, but does not seem to be a decisive factor in the general trend of fluctuation.

On what cause or combination of causes does the autumnal fall in mosquito numbers depend? The only known and observable ecological factor whose variation coincides with it and parallels it is the number of daylight hours. The difference between solstice in June and equinox in September is 53 minutes 37.5 seconds in the Motagua. This factor is related to latitude, and we know that it is negligible closer to the equator where we do not observe any spectacular diminishing of mosquito density during the fall. In the absence of controlled observations which the program of this work could not include, all that can be done

here is to point out the trend of the phenomenon, taking into consideration its epidemiological implications.

It should be pointed out that the amounts in Figure 2 are the result of captures made with clothed human bait in the canopy; as such, they do not express the attraction these strictly specialized canopy mosquitoes may feel toward their usual staple, the canopy mammals. There are reasons to believe that a monkey roaming the foliage stirs up and attracts a much greater number of blood-sucking anthropods than a man clinging to a limb or sitting on a platform. Occasional close range observations of monkey bands in the canopy have shown that they are usually besieged by swarms of culicines and other diptera. It has been shown experimentally (Boshell and Osorno Mesa, 1944) that *Haemagogus* mosquitoes can follow their prey patiently and for a long time until given a chance to bite. In several instances the bodies of monkeys recently shot down during immunity surveys were seen to be attacked while being bled by *Haemagogus* mosquitoes which had evidently followed them down from the canopy, in places where ground captures were uniformly negative. These remarks may help to explain the apparent discrepancy observed in certain sectors between relatively high monkey mortality rates and low yields in mosquito captures.

Virus isolations. As seen in Table 3 the virus isolations from mosquitoes can be summarized as follows:

Species	Place	Yellow fever	Ilhéus virus	Other virus
H. mesodentatus	Río Blanco	11		
	Tipón	3		1
H. equinus	Río Blanco	2		1
	Tipón	1		
S. chloropterus	Río Blanco	3	1	
	Tipón	1		
Total		21	1	2

Total by localities:

Place	Yellow fever	Other virus	Total
Roí Blanco	16	2	18
Tipón	5	1	6
			24

In addition to the positive results listed in Table 3 and above, a total of 2,767 *H. mesodentatus*, 1,418 *H. equinus*, and 2,724 *S. chloropterus* were captured during the same period and upon inoculation gave negative results.

It is evident that the virus persisted on the slopes of the Montaña del Mico at least from February to August. The last monkey mortalities were seen in that sector in May. The virus seems to have survived in mosquitoes for over two months after the last infected monkey was detected. This is approximately the assumed life span of *Haemagogus* in nature. The fact that virus was recovered repeatedly from three mosquito species constitutes fairly strong circumstantial evidence that they play a role in transmission of the disease. We are aware, of course, that the only final and definite proof that a mosquito can transmit a virus by bite in nature consists in a positive transmission by bite "in situ", such as was obtained on other occasions in Colombia and Brazil. The available facilities did not allow us to attempt any such experiment. Nevertheless, in the present case, it is felt that the fortunate concordance of laboratory transmission results with numerous virus isolations from the same species in nature permits one to draw eipdemiological conclusions.

Primate mortality. The remarkable contrast which exists between the Motagua Valley and other regions in the Northern Coast of Honduras should be pointed out at this stage.

In the Masica area in Honduras, we saw large numbers of monkeys survive after the 1954 episode. Eighteen months later virus was active again a few miles to the west near Esparta. In a similar way large numbers of monkeys have survived the outbreak of 1956 in Esparta, leaving all kinds of possibilities open for the future. In the Motagua Valley, on the contrary, the primate population appears to have been virtually wiped out. In several months, only one group of howlers has been reported from a place called El Jute, midway between Río Blanco and El Tipón. People in the valley cannot remember when they last heard the familiar howling concerts. The last *Ateles* were seen in July in Río Blanco, a lonely small group.

The difference appears to be related to the characters of the vectors involved. In the northern plains of Honduras, only *H. equinus* and *S. chloropterus* are to be found (Trapido and Galindo, 1955). In the Motagua Valley, *H.*

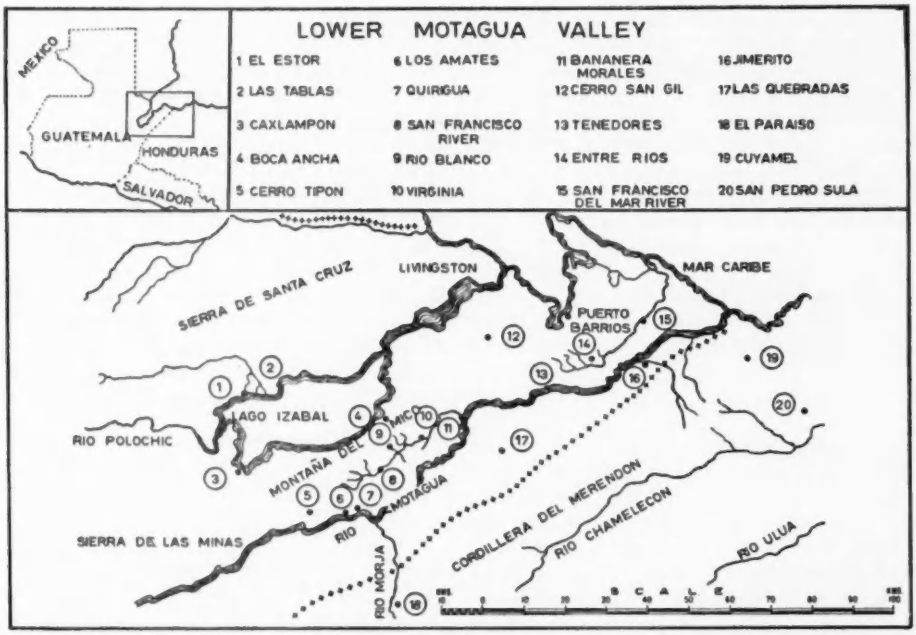

FIG. 3. Sketch map of the lower Motagua Valley showing area where study was conducted. Numbered locations are mentioned in the text. Insert map shows relation of lower Motagua Valley to neighboring middle American countries.

mesodentatus is prevalent. It is a vicious and aggressive species and seems, from the proportion of virus isolations, to have a greater affinity with yellow fever virus than its two associates.

It thus appears that a distinction has to be established between regions with *H. mesodentatus* and regions without *H. mesodentatus*. In the first, outbreaks are promptly evidenced by high mortality, the primates are eliminated as a reservoir by death or immunization and the region may be considered free of infection three months after the last monkey mortality is observed. In the second, the process may lurk, undetected, for a long time; the primate reservoir, whittled down slowly and inconspicuously, may last for a protracted period. These facts should be taken into account before pronouncing a region free of yellow fever.

The extension of the wave. Simultaneously with the work in the Motagua Valley, surveys were conducted in several directions with the double purpose of detecting eventual invasions of new territories and of studying in advance the ecological conditions of the threatened areas. These surveys established the fact that in all the forested regions situated to the north and to the west of the Motagua Valley, that is, in the Alta Verapaz and the Petén, the association of primate with mosquito vector is to be found. By primate we mean the two species *Alouatta palliata* and *Ateles geoffroyi*; by mosquito vectors, the three species *Haemagogus mesodentatus*, *Haemagogus equinus*, and possibly *Sabethes chloropterus*. Another *Haemagogus* sub-species, *Haemagogus mesodentatus alticola*, new to science (Galindo and Trapido, 1956), is found along the foothills of Alta Verapaz and on into Chiapas, Mexico. This may introduce another factor whose consequences as yet cannot be determined.

During the past year this enzootic has progressed in several directions. The right bank of the Motagua was invaded at almost the same time as the left, and consequently, following the valley of the Morja river, the virus re-entered Honduras in the region of El Paraiso. The southern shores of Lake Izabal were involved as early as February near Bocancha. The virus advanced along the piedmont in a southwesterly

direction, reaching the end of the lake near Caxlampón in the first week of May. By the middle of August, monkeys were dying on both banks of the Polochic river. In September and October, the mortality extended to the northern shore of the lake (Estor, Las Tablas) and the foothills of the Santa Cruz range.

The virus invasion has thus reached the territories situated north of Lake Izabal. The road is open, with solid forest continuity and with the primate-mosquito association, toward the foothills of Belice, the Alta Verapaz, the gallery forests of the Petén and from there, to the states of Chiapas, Tabasco and Campeche of Southern Mexico.

SUMMARY

With the crossing of the yellow fever epizootic wave from Honduras to Guatemala at the end of 1955 a new ecological area with unknown vectors became involved. Starting in March and continuing until three negative months had been recorded, mosquitoes were captured in the lower Motagua Valley and sent to the Gorgas Memorial Laboratory in Panama where attempts at virus isolation were made by inoculation of species lots into various mammals. During the course of the studies 25 viruses were isolated—22 of yellow fever and 3 of other viruses. As a result *Haemagogus mesodentatus* was shown to be the prime vector with *H. equinus* and possibly *Sabethes chloropterus* as secondary transmitters. The epizootic wave after decimating the primates of the lower Motagua Valley has continued across the Lake Izabal area and is now headed for Petén and Alta Verapaz, areas suitable for the continuation of the disease into southern Mexico, where favorable conditions for sylvan yellow fever exist.

ADDENDUM

In February 1957 evidence of virus in the form of positive monkey livers has been obtained in the S.W. Petén as far west as the forests of the Salinas River, which is the Guatemalan-Mexican border. In May, positive livers were found near Lake Petén and in Mountain Cow, District of El Cayo, British Honduras.

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VIRUS ANTIBODY SURVEY ON SERA OF RESIDENTS OF THE AMAZON VALLEY IN BRAZIL

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The virus antibody studies reported here were carried out in the Amazon Valley in cooperation with the Brazilian *Serviço Especial de Saúde Pública* (SESP) as a part of The Rockefeller Foundation's cooperative program of research on arthropod-borne (arbor) viruses throughout the world. In August and September, 1953, blood samples were collected in 15 localities that had been selected as representative of the region and were readily accessible by automobile, plane or launch; most of these places were served by SESP health posts. During visits of from two to five days, blood was taken from adult residents who had spent all or most of their lives in each particular locality. A second survey was made in six of these areas in June, 1954, to ascertain the distribution of antibodies among children. In a third survey, made in July, 1954, along the forested banks of the infrequently traveled Capim River, blood samples were taken from residents representing all age groups and both sexes. Mouse protection tests on the 509 sera from these surveys were performed in the Rockefeller Foundation Virus Laboratories in New York.

COLLECTION OF SERA

The sera collected in or near Belém were obtained from five groups of donors. The first group, designated as BE in Table 1, consisted of 16 workers at the Virus Laboratory who had lived in the city for more than 10 years. The second (BS) comprised 35 children in one of the public schools in Belém. In the third group were 76 workers at an agricultural experiment station about five kilometers from the center of the city, situated in the rain forest where many men were employed as road builders and woodcutters. The fourth group was made up of 48 residents of Castanhal and Apeú, villages about 70 kilometers from Belém in which an SESP Health Post and Sub Post, respectively, are located. The fifth group consisted of 37 lumberers and workers at a sawmill in a virgin forest about 10 kilometers beyond Castanhal.

The other areas surveyed were all river towns on the Amazon or on its tributaries. The town nearest Belém was Abaetetúba on the river Abaeté, five hours distant by motor launch. The land here is an alluvial delta planted to sugar cane, which furnishes the principal industry of the region in the distillation of alcohol and rum. The people are progressive, industrious and generally better nourished than the residents of many other interior towns which depend solely on the export of natural products. A total of 58 blood samples was collected in Abaetetúba.

Cametá is situated on the left margin of the river Tocantins, about 12 hours by launch from Belém. This is an old and decadent town which in its best days was for a time the capital of the State of Pará and the center of rich plantations of cocoa. Now it is merely a port for rubber and uncultivated nuts. Forty-one samples of blood were obtained here.

Altamira on the river Xingu is most conveniently reached by hydroplane; rapids just below the town prevent navigation except by small sailing craft. It is a collecting port for rubber and nuts from the upper Xingu. Produce is transported by truck around the rapids to the port of Victoria. This interrupted navigation has delayed exploitation of the region to such an extent that unfriendly Indian tribes live in close proximity to the village. Twenty-four specimens were collected in Altamira.

Óbidos is situated on the left bank of the Amazon, at the narrowest point in the main channel. It is the site of an old fort, which is still actively maintained, and enjoys a moderate commerce in forest products and animal skins. Only 14 samples were collected here.

Santarem is the third largest city in the Amazon Valley and lies at the junction of the river Tapajoz with the Amazon on high sandy flat terrain. SESP maintains a hospital and training center for auxiliary nurses here in addition to the Health Post. A total of 19 samples was collected.

TABLE 1

Results of neutralization tests with sera from the Amazon Valley

		EEE	Semliki	VEE	WEE	Dengue I	Dengue II	Ilhéus	St. Louis	Uganda S	West Nile	YF	Zika	Bwamba	Bunyamwera
BS Belém	C*		0/35					1/35							
BE	A†	0/16	0/16	0/2		2/13		1/16	2/16	0/16			0/16		
Agricultural	C		0/25					0/25							
Station	A	3/47	8/51	1/2	0/2	1/38		17/51	7/47	0/28	0/1		1/28		4/26
Castanhal	C		0/22					0/22							
Apeú	A	0/26	0/26		0/26	5/17	1/26	4/26	3/25	1/13	1/13	4/10	0/13		0/26
Sawmill	C		0/15					0/15							
	A	0/19	9/22	4/19	0/19	1/15	0/19	10/22	1/19	0/9	0/9	5/10	0/9		0/19
Abaetetúba	C		1/22					2/22							
	A	1/31	5/36		0/5	2/23	0/5	19/36	0/31	0/13	0/10	3/10	0/13		1/19
Cametá	C	3/12	0/12		0/12			3/12							
	A	9/29	7/29	0/6	3/28	4/28		17/29	3/28	1/18	1/10	10/10	3/18		1/28
Altamira	A	0/24	1/24			1/23		9/24	0/24	0/3	0/8	3/7	0/3		1/21
Óbidos	A	0/14	2/14			1/14		4/14	1/14		0/5	2/9			0/3
Santarem	A	0/19	0/19			1/19		5/19	0/19		0/15	0/3			0/10
Fordlandia	A	0/11	1/11			1/11		2/11	0/11			1/8		0/11	0/11
Belterra	A	0/17	5/17	0/1	0/1	3/17		7/17	0/17		1/14	1/3		0/3	0/3
Manaus	A	0/15	1/15			2/15		2/15	1/15		1/5				
Lábrea	A	0/24	7/24			2/24		8/24	1/24		2/9	9/15		0/15	0/24
Tefé	A	0/22	3/22			4/22		9/22	0/22			17/15		0/11	2/22
Capim	C		0/19			0/19		1/19	0/19			3/19			0/19
	A		1/33			0/33		16/33	0/32			14/32			2/32

* Children.

† Adults.

Fordlandia and Belterra on the right bank of the river Tapajoz were visited by launch from Santarem. These are old Ford rubber plantations which now are owned by the Brazilian government. SESP does not maintain posts here. A total of 28 samples was obtained from these two places.

In the State of Amazonas, blood samples were collected in the three towns of Manaus, Lábrea, and Tefé. Manaus, on the Rio Negro near its junction with the Amazon, is the State capital and second only to Belém in size; fifteen samples were collected here. At Lábrea, a rubber port on the River Purús, 24 samples were obtained, and at Tefé, on the Solimões or Upper Amazon, 22 samples.

A five-day launch trip was made on the Capim River, which flows in a sinuous course of 1,000 kilometers through forested, sparsely settled country in the State of Pará. The residents live on the river bank in isolated houses or in small groups of related families and are more nearly self-sufficient than the residents of most large river communities. The men go out in groups to hunt and fish and cut lumber for exportation down river in large rafts, while the agricultural products used are grown by their families. The residents examined professed not to have traveled to other municipalities and rarely even to the village of Capim. The 52 samples collected here proved to be of special interest in the serological survey, as will be discussed later.

RESULTS OF NEUTRALIZATION TESTS

Mouse protection tests (Bugher, 1940) on the survey sera were performed in the New York laboratories. Results are summarized in Table 1. A total of 509 persons between the ages of four and 77 years were examined, of whom 150 were children 14 years of age or less. Sera were obtained from children in only seven of the localities.

Fourteen arbor viruses were employed in the protection tests with these sera, as follows: Eastern, Venezuelan and Western equine encephalomyelitis, Semliki Forest, dengue I and II, Ilhéus, St. Louis encephalitis, Uganda S, West Nile, yellow fever, Zika, Bwamba and Bunyamwera.

Antibodies capable of neutralizing all these agents, with the exception of Bwamba, were found to be present. The numbers of sera tested against the different viruses varied greatly, however. For example, all the specimens were tested against the Ilhéus and Semliki Forest viruses, but only 50 were tested against the Bwamba virus. The results obtained with each virus will be discussed briefly.

Eastern equine encephalitis. Of 314 adult sera tested, 13 were protective. The donors of these positive sera were residents from the neighborhood of Belém, Abaetetúba and Cametá. The incidence of immunity in Cametá was surprisingly high: 3 of 12 sera from children and 9 of 29 adult sera were protective. It would appear that the neighborhood of Cametá provides an unusually favorable environment for EEE virus. In spite of this high rate of immunity, there is no history of any unusual incidence of encephalitis. It will be recalled that in the United States, infection of man with this virus is characterized by severe encephalitic symptoms and a high mortality rate. It is of interest that recently several strains of EEE have been isolated from sentinel monkeys near Belém.

Semliki Forest virus. All the survey sera were submitted to neutralization tests with this agent. Sera giving positive results came from areas located throughout the entire Amazon Valley. At first, results were interpreted to mean that the Semliki Forest virus was present in the Amazon Valley. Considerable doubt has been cast upon this interpretation, however, by the discovery both in Trinidad and on the Guamá River near Belém of the Mayaro virus, an agent closely related immunologically to Semliki (Anderson *et al.*, 1957; Causey and Maroja, 1957; Casals and Whitman, 1957). Very good evidence has been obtained that the positive results obtained in the neutralization tests with the Semliki virus are due to antibodies produced by Mayaro virus infection. This evidence is discussed in greater detail in an account by Causey and Maroja (1957) of an epidemic caused by the Mayaro virus.

Venezuelan equine encephalitis. Of 30 adult sera tested with this agent, 5 were found to be protective. The donors of the positive sera were workers at the agricultural experiment station near Belém and the sawmill near Castanhal. The validity of these findings was confirmed by the repeated isolation of strains of VEE virus from man, sentinel monkeys, and mosquitoes in localities near Belém.

Western equine encephalitis. Of 80 adult sera tested, 3 were found to be protective against WEE virus. The donors of these positive sera were residents of Cametá. It seems likely that these positive results are due to infection with WEE virus. The possibility must be considered, however, that the results are due to immunological overlap with other group A arbor viruses. Although, in general, the degree of immunological overlap in the group A viruses is not as marked as in group B, recent observations by Casals (1957) have shown clearly that marked overlap may occur in experimental animals. To date, no strains of virus identifiable as WEE have been isolated in the Amazon Valley.

Dengue. Since the results of preliminary neutralization tests with the two strains of dengue indicated that the incidence of antibodies to dengue I was higher than the incidence to dengue II, subsequent tests were performed with dengue I. The results are summarized in Table 2. It will be seen that the donors of sera giving positive results are all adults. All of 50 sera obtained from individuals 15–19 years were negative. In fact, no person less than 25 years of age showed antibodies to dengue. These findings suggest that, although at one time it was undoubtedly present in the region, human infection with dengue virus is now probably absent. It is of interest to compare these results with those obtained in a similar survey in Trinidad (Downs *et al.*, 1956). In the latter region, the incidence of immunity to dengue I is high not only in the capital city, Port-of-Spain, but also in rural, densely forested areas. The difference between Trinidad and the Amazon Valley is no doubt a reflection of the abundance of the mosquito *Aedes aegypti*—a common vector of dengue. At the time of the immunity survey in Trinidad, this mosquito was very abundant and widely dispersed on the island. *A. aegypti* was once present in the Amazon Valley but has now been completely eradicated from the area.

Ilhéus. Neutralization tests with Ilhéus virus were done on all 509 sera collected in the survey. Among 150 children between 4 and 14 years of age, 7, or 4.7 per cent, were found to be positive. The 359 sera in the age group 15–77 years showed an incidence of 36.2 per cent positive (Table 3).

TABLE 2

Distribution by age groups and sex of the results of neutralization tests with dengue I virus

	Male			Female			Grand total	Number positive	Per cent positive
	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive			
15–19	26	0	0	24	0	0	50	0	0
20–29	68	2	2.9	36	1	2.8	104	3	2.9
30–39	60	14	23.3	23	1	4.3	83	15	18.1
40–49	25	2	8.0	18	4	22.2	43	6	14.0
50+	19	5	26.3	8	1	12.5	27	6	22.2
	198	23	11.6	109	7	6.4	307	30	9.8

TABLE 3

Distribution by age groups and sex of the results of neutralization tests with Ilhéus virus

	Male			Female			Grand total	Number positive	Per cent positive
	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive			
15–19	37	7	18.6	32	5	13.6	69	12	17.4
20–29	78	31	39.7	46	16	34.8	124	47	37.9
30–39	65	28	43.1	30	6	20.0	95	34	35.8
40–49	26	14	53.8	18	4	22.2	44	18	40.9
50+	21	14	66.7	8	5	62.5	29	19	65.5
	227	94	41.4	134	36	26.9	361	130	36.0

There was a significantly higher incidence among males (41.4 per cent) than among females (27.3 per cent). It is apparent that infection with the Ilhéus virus is very prevalent and widespread in the Amazon Valley. The higher incidence of immunity in males is due, in all probability, to the fact that Ilhéus infection is acquired through close contact with the forest.

St. Louis. There were 19 St. Louis positives (5.5 per cent) among 344 sera tested. These were distributed throughout the Valley. The interpretation of these results is difficult, owing to the known immunological overlap between members of the group B arbor viruses. Ten of the positives occurred with Ilhéus or dengue antibodies or both, and nine occurred in the absence of antibodies to these two agents. Since the St. Louis virus has been isolated in Trinidad, a neotropical region, it is probable that this virus occurs also in the Amazon Valley, which is likewise neotropic. The results of the neutralization tests with Amazon sera are consistent, though not conclusive, evidence that infections with the St. Louis virus occur in the Amazon Valley.

Yellow fever. Yellow fever was the only infection of this arthropod-transmitted group previously known to exist in the Amazon region of Brazil. Although the virus has been isolated only recently, diagnosis of yellow fever had frequently been made on the basis of pathological lesions in liver specimens from fatal cases of fever in man. Yellow fever vaccination campaigns have been carried out in all of the States and Territories in the area on various occasions. The usual stimulus for these is the report of fatal cases of yellow fever. The area visited for vaccination is comparatively small in relation to the region involved, and the population treated is very small in comparison with the vaccination programs undertaken in more populous southern Brazil. During the years 1951 to 1953, 136,000 vaccinations were made by the National Yellow Fever Service in the Amazon Valley. It was realized, therefore, that the neutralization test for antibodies to this virus might not give a true picture of previous infection and consequently, fewer tests were performed than originally planned. The results on the sera from the River Capim, however, where

vaccinations had not been undertaken, are assumed to give information on the natural infection rate in a forest area. Here, 14 out of 32 adults examined, or 43.8 per cent, gave positive protection tests for yellow fever; the sera of 3 out of the 19 children under 14 years of age, or nearly 16 per cent, were also positive. As it happened, of the total number of adult sera from all areas tested for protective antibodies against yellow fever, 44.7 per cent were positive, a figure very close to that found for the adults examined in the Capim River area (Table 1).

Sera from children in areas other than the Capim River have not been tested for yellow fever antibodies. A total of 132 sera collected from the adult group 15 to 77 years of age were tested for protective antibodies for yellow fever (Table 4). Of these, 72 were from males and 60 from females. The males showed a higher rate of previous infection, namely, 54.2 per cent, as compared with 33.3 per cent for the females. This difference in rate may be due to the greater occupational exposure of the males. Analysis by age groups showed no marked differences (Table 4). The results are in accord with the habits of the people in this region where the adolescent takes his place beside his elders in the work of collecting forest products, clearing land for planting and hunting or fishing along the jungle rivers. The women and girls are often included in these activities, especially when the wild nuts and fruits are harvested or when the small clearings in the jungle are being worked.

The occasional outbreak of yellow fever in the Amazon Valley is not a cause for alarm among either the inhabitants or the health authorities. This situation is the result of several factors peculiar to the region. In the first place, nearly half of the adult population is immune, either by naturally acquired infection or by yellow fever vaccination, and the extent of the outbreak and its severity in any given region are therefore limited. A second factor is the absence of *Aedes aegypti*, which means that the susceptible individual must either visit the forest or live in close proximity to it to acquire his infection from sylvan mosquitoes. Last, but perhaps not least, is the perpetual presence of the virus in the monkey-mosquito population, with no natural barriers to curb transmission. This statement should not be taken to imply that the virus is always present in any given place. The virus may be imagined as constantly shifting in its enormous habitat, appearing and reappearing as sufficient host susceptibles and vectors permit. This enzootic condition is reflected in low mortality rates among the simian tribes. Close questioning of many hunters and woodsmen in the Belém area, for instance, elicits no stories of dead or sick monkeys in the forests. The ecological picture is in marked contrast with the epizootics and epidemics of yellow fever that periodically sweep from the Amazon through central and southern Brazil, where monkey populations may be decimated and sometimes exterminated in the small, isolated forests, and death from yellow fever is noted among the human inhabitants.

Other group B viruses. There were 6 West Nile positives among the 99 sera tested, and all 6 were positive for one or more other group B viruses. Ilhéus antibodies were common to all but one, and the exception was positive for dengue. The 4 sera positive for Zika, among the 100 sera tested, were also positive for several other group B viruses. Neither of the 2 sera positive for Uganda S among the 96 tested was

TABLE 4

Distribution by age and sex of the results of neutralization tests with yellow fever virus

	Male			Female			Grand total	Per cent positive
	Number tested	Number positive	Per cent positive	Number tested	Number positive	Per cent positive		
15–19	10	5	50.0	11	2	18.2	21	33.3
20–29	21	10	45.5	18	5	27.8	39	38.5
30–39	22	11	50.0	16	4	23.5	38	39.5
40–49	9	8	88.9	13	7	53.8	22	68.2
50+	10	6	60.0	2	1	50.0	12	58.3
	72	40	55.6	60	19	31.7	132	44.7

examined for yellow fever antibodies. One of them, however, was positive and the other negative for other group B viruses tested. The decision as to whether West Nile, Uganda S or Zika virus occurs in the Amazon Valley must be left in abeyance. It is possible that the positive sera to these agents are due to the marked immunological overlap among members of the group B viruses.

Miscellaneous viruses. Other viruses tested with the Amazon sera were Bwamba and Bunyamwera. None of 50 sera examined showed protective antibodies for the former, while 11 of 232, or 4.7 per cent, of the sera tested showed neutralization of Bunyamwera virus. The latter agent is immunologically unrelated to any other known arbor virus and, consequently, the positive findings are interpreted as clear evidence that a Bunyamwera-like virus occurs in the Amazon Valley.

SUMMARY

Sera from residents in 15 localities in the Amazon Valley were examined for antibodies to 14 arbor viruses by neutralization tests in mice. Viruses neutralized in high ratios were yellow fever, 44.7 per cent; Ilhéus, 36.2 per cent; VEE, 16.7 per cent; and Semliki Forest virus, 13.9 per cent. Viruses neutralized in lower ratios were dengue I, 9.6 per cent; West Nile, 6.1 per cent; St. Louis, 5.5 per cent; Bunyamwera, 4.7 per cent; EEE, 4.1 per cent; Zika, 4.0 per cent; Uganda S, 2.1 per cent; dengue II, 2.0 per cent and WEE, 1.6 per cent. Bwamba was the only virus tested for which no antibodies were found. The evidence is not conclusive that Uganda S, Zika, West Nile, St. Louis, dengue II, or WEE virus occurs in the Amazon Valley. The positive results obtained with these agents may be due to immunological overlap with other immunologically related agents.

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EYE INFECTIONS IN A PUNJAB VILLAGE

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Epidemiologic field studies of eye infections in a Punjab village were done to define some of the factors influencing prevalence in a hyperendemic area.

Few diseases of the tropics cause as much mass disability and distress as eye infections. Thus far research has been oriented mainly toward clinical definition and therapy. Trachoma is the diagnostic label applied to what appears to be a very common but variable clinical entity. Little has been learned about trachoma either epidemiologically or as a result of laboratory studies; etiology is as yet unproved although there is scattered evidence from limited volunteer studies that the agent may be filterable and a virus (Thygeson and Richards, 1938). The only laboratory procedure of practical use has been finding inclusion bodies in early cases, but until the virus is isolated it is difficult to evaluate the significance of these bodies.

Some surveys have been done on selected groups such as patients in clinics, soldiers, and school children. (Bietti, 1951; Braff and Winkelstein, 1952; Chang, 1951; Scott, 1950; Murray, 1953). These surveys have been used in guiding control programs and they have given certain epidemiological impressions. In some studies the numbers examined included thousands of people, but the samples were restricted to limited and often unrepresentative groups.

For a more complete picture of the epidemiology of eye diseases it is better to study either a total population unit such as a village or a statistically representative sample of a population. In the few field studies in total population units which have been reported it has been possible to study fundamental epidemiological questions such as whether infections are acquired in homes or at school, by gathering basic data on the age distribution of infected persons.

The best known study of trachoma prevalence was made by Wilson (1929) in Egypt almost 30 years ago. Bahtim village was on a royal estate where the people were under good control. Clinical examinations of 3,058 of the total population of 3,549 were made. Trachoma was diagnosed in 25 per cent of children 0–1 years old, 64 per cent of those 1–2 years old and thereafter the rate ranged from 84–99 per cent. It was apparent that infections were occurring primarily in the home. Weir and his co-workers (1952) in Sindbis village found a somewhat similar distribution of trachoma. They focused their attention primarily on acute bacterial ophthalmias caused by Koch-Weeks bacilli and gonococci and showed that fly control can diminish seasonal spread, especially of gonococcal infections. Thygeson and Forster (1939) examined the eyes of all 630 residents of two small Apache villages and found infection rates ranging from 12–34 per cent for various age groups with children's rates being twice that of adults. In 23 per cent of the total of 165 families both parents and children had trachoma while in 13 per cent of the families only children had trachoma. This suggests that infection was occurring both in and out of the home. Tabone (1951) reported on examinations of almost 17,000 of the 28,000 people living on the island of Gozo near Malta. Of the 4,000 school children, 17 per cent had clinical trachoma and 7 per cent conjunctivitis while only 5 per cent of the persons examined in the general population were infected. He concluded that this indicated that infection was occurring mainly among school children.

With the availability of modern mass treatment methods it has become increasingly important to try to discover the age and probable place where eye infections are acquired. It is easy to treat school children and where infections are occurring primarily at school age this is logical. Where infection rates in preschool children are high the rationale of school campaigns is doubtful and certainly campaigns should not be based on experience from areas where the epidemiological pattern is quite different. The examples cited above suggest that the principal site of spread of eye infection was in the home in a hyperendemic area; in both preschool and school

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children in an area of moderate endemicity; and largely among school age children in an area of relatively low endemicity.

PROCEDURE

In 1953 as part of the research and teaching program of the Ludhiana Christian Medical College, Punjab, India, four village health centers were established. Complete census data were collected, maps and surveys of sanitation and nutrition were made. Sarinh village was selected for preliminary studies of eye infections. Our studies were limited to school populations until July, 1955 when a house-to-house survey of eye infections in the population was done by two doctors who lived in the village for two weeks. Since we wanted to get basic data on as many people as possible rather than detailed data on a few, we concentrated on clinical examinations. We took conjunctival scrapings early in the survey to verify diagnoses, but found that crying babies made us unpopular.

Until the etiology of trachoma is clarified and laboratory tests developed, it is hazardous to attempt clinical classification. With increasing evidence that adenoviruses and perhaps other viruses can produce follicular conjunctivitis which might be confused with trachoma (Symposium, 1957), it is possible that the conditions which have been called trachoma may be due to etiological complexes in which several agents act together. Added to the possible virus combinations are the effects of several bacteria which are known to cause disease or which may work synergistically with other agents in causing disease. It is probable that some of the severe complications of traditional trachoma may be due largely to associated bacterial infections.

In this paper, therefore, we have used the general term "eye infections." It should be clearly stated, however, that over 90 per cent of the cases we saw had characteristics which would have been considered typical of trachoma by most eye specialists. Pannus, entropion with

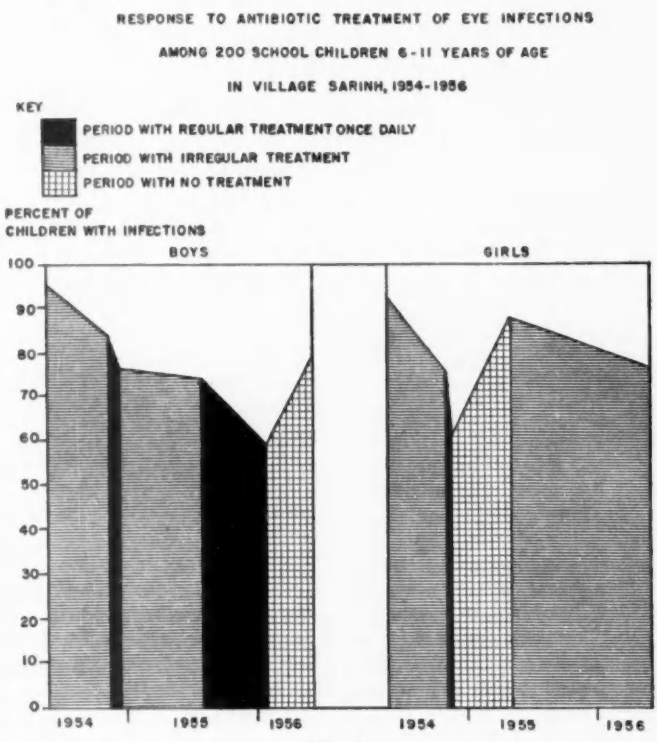


FIG. 1

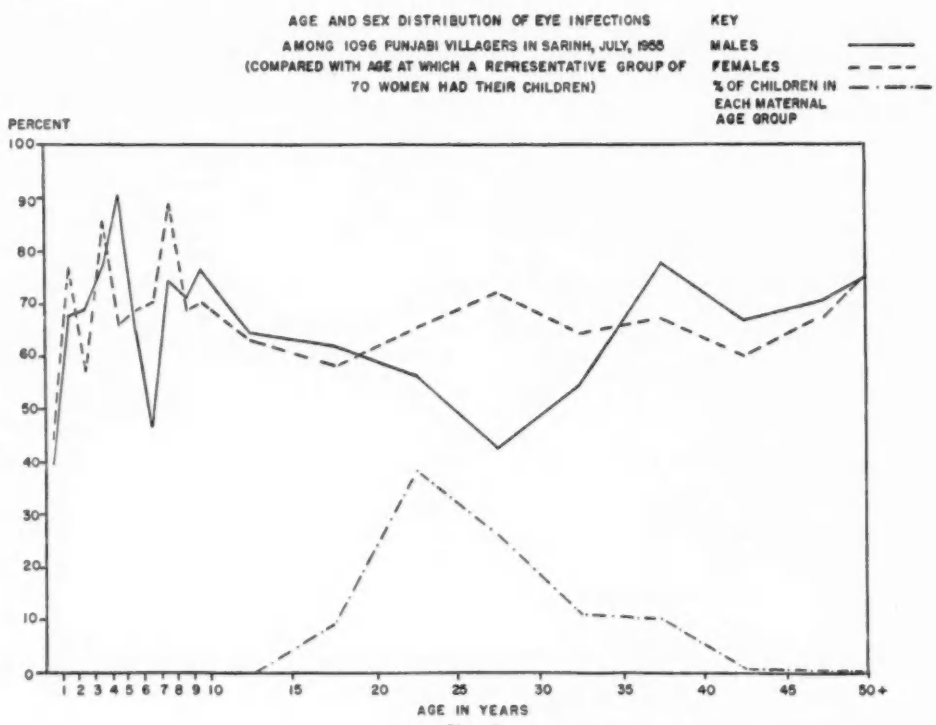


FIG. 2

trichiasis, corneal scarring and other late stigmata were common.

RESULTS

1. *School surveys.* Surveys of all school children in the two primary schools in Sarinh village were carried out periodically from 1954–1956. The enrollment of each school ranged around 100 children aged 6–11. At the start eye infection rates were 96 per cent in the boys' school and 91 per cent in the girls' school. Teachers were taught how to apply an antibiotic ointment[2] and a procedure of having the children line up for instillation of medicine once a day was worked out. Symptomatic relief was so prompt that it was difficult to get regularity of treatment sufficient for clinical cure. Figure 1 shows that during periods when we relied on teachers alone for treatment the number of clinical cures increased slowly. When the "health visitor" who lived in the village personally checked on daily treatment to ensure regularity there was a more rapid drop in rates. As soon as treatment was stopped, however, infection rates quickly returned to high levels. The clinical severity of symptoms also returned abruptly. These findings and the continuing number of cases seen in the dispensary suggested that in Punjab villages a mass treatment program in schools alone would reach too small a segment of the infected population to contribute significantly to control.

2. *General population survey.* In July, 1956 the available population of Sarinh was examined ophthalmologically by two doctors working together to check each other's opinion. This was about 10 weeks after the close of school and therefore the stopping of treatment in schools. Village people were seen at home or in their fields and other places of work. According to our census the population of Sarinh was 1,342. We examined 1,096. Of the remaining 246 persons 77 per cent were adults, mostly men who were away in the

[2]Tetracyn by courtesy of Chas. Pfizer & Co., Inc., Brooklyn 6, New York.

city working. Little is known about their infection rate, but presumably it was somewhat lower than the village average. All calculations, however, were necessarily based on the 1,096 people examined, since they were the ones actually available in the village at that time.

Age and sex distribution of eye infections. Figure 2 gives the age and sex distribution of eye infections. Of the children under one year of age, 49 per cent had eye infections. Of the five children under one month, two had eye infections. Rates for one-year-old children rose to 75 per cent. The male peak of 91 per cent was reached at four years and the female peak of 90 per cent was reached at seven. It is of interest that boy babies have a great deal of attention lavished on them and are handled more than girl babies. Once they are able to get out and play, however, they manage to get away from their grandmothers and aunts. This may explain the different ages of maximum incidence. Infection rates in adulthood were essentially level at 60–70 per

TABLE 1

Per cent of eye infections in adults and children of 225 families in Sarinh, July, 1955

Children	Adults				
	Number of families	Both parents infected	Only mother infected	Only father infected	Both free of infections
Number of families	225	97	64	26	38
All children in family infected	65	35%	28%	19%	21%
Some children infected	90	43%	44%	27%	34%
None of children in family infected	70	22%	28%	54%	45%
Total %		100%	100%	100%	100%

P = > .02.

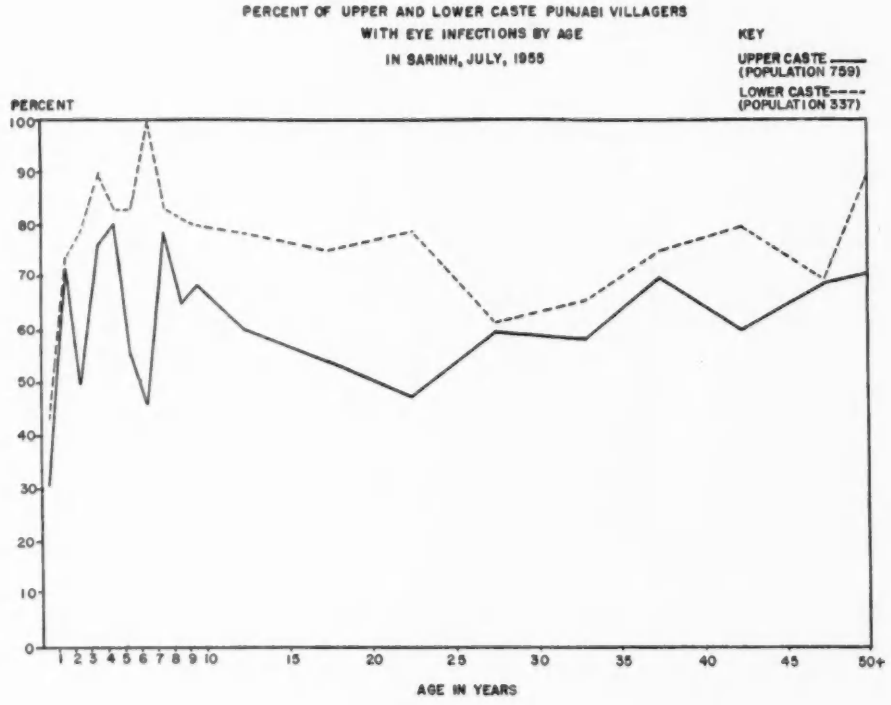


FIG. 3

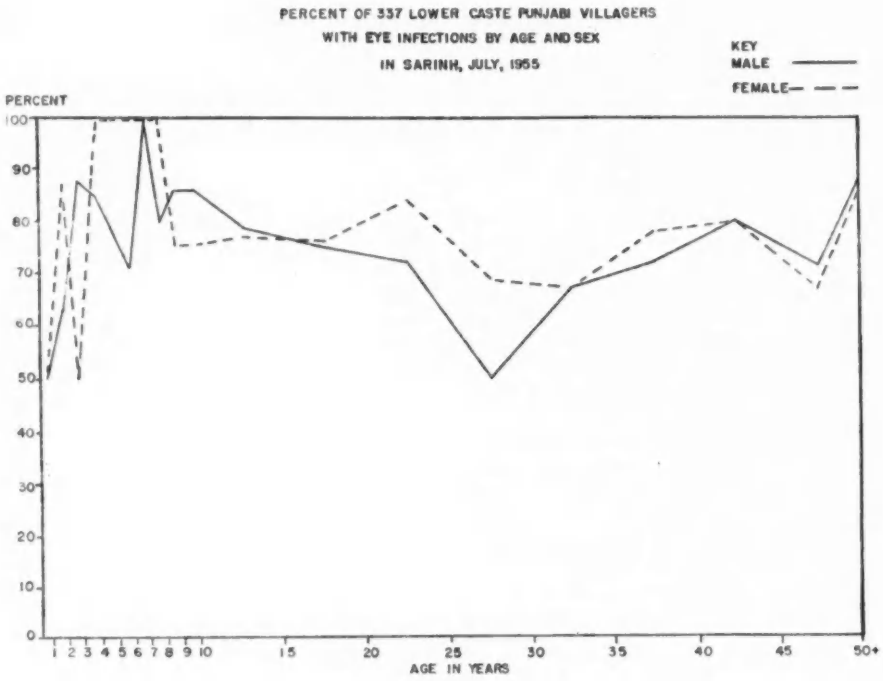

PERCENT OF 337 LOWER CASTE PUNJABI VILLAGERS
WITH EYE INFECTIONS BY AGE AND SEX
IN SARINH, JULY, 1955
KEY
MALE
FEMALE
PERCENT
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 15 20 25 30 35 40 45 50+
AGE IN YEARS

FIG. 4A.

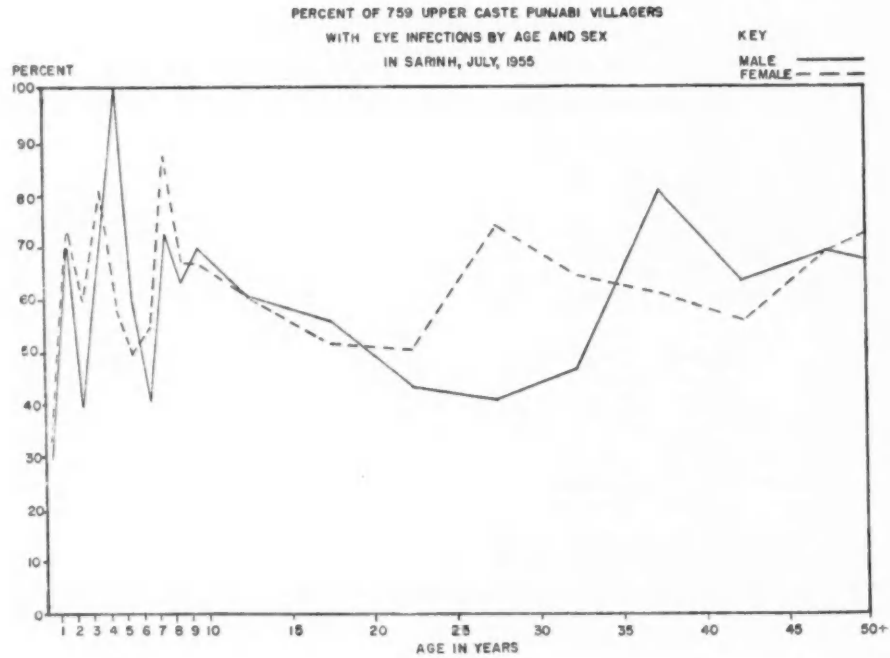

PERCENT OF 759 UPPER CASTE PUNJABI VILLAGERS
WITH EYE INFECTIONS BY AGE AND SEX
IN SARINH, JULY, 1955
KEY
MALE
FEMALE
PERCENT
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 15 20 25 30 35 40 45 50+
AGE IN YEARS

FIG. 4B.

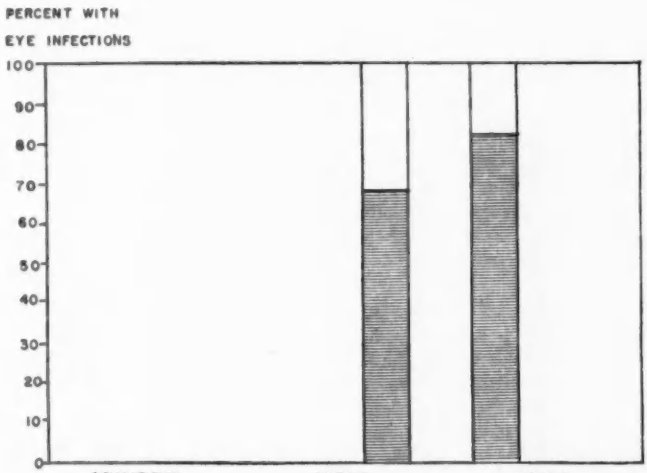
cent. The overall average prevalence rate for all age groups was 73 per cent.

Of considerable practical interest is the divergence of male and female rates in the age span 20–35 years. A chi-square test of the statistical significance of this shift in male and female rates indicates that this difference is significant with a P of less than .01. It is of interest that this period when female rates rise is also the period when women are most involved in bringing up their children. Also shown in Figure 2 are data from another study giving the number of children born to a representative sample of 70 village women at various ages. It can be seen that the peak of this curve is a few years earlier than the peak of the trachoma curve which would coincide with the time when children were having their highest infection rates.

The relationship between infections in mothers and children was brought out more clearly by analyzing the data by families. There were 225 households in the village. In many instances these were joint families in which two or more biological families lived as a single epidemiological unit. Table 1 shows the association between infection of the parents and children. It is apparent that when both parents or when mothers only were infected there was a high rate of infection among children, while if neither parent or the father only was infected there was a much better chance that all children would be free of infection. This is significant with a P of less than .02.

Caste distribution of eye infections and socioeconomic status. In a general way the caste structure of a village indicates socioeconomic status. In order to avoid distinctions which might be subject to variation, caste groups have been divided into upper caste and lower caste. The upper caste groups are the Jat Sikh farmers and the Hindu castes of Brahmins, shopkeepers and tradesmen. The lower castes include leather

PREVALENCE OF EYE INFECTION IN TWO LOWER CASTE AREAS WITH CONTRASTING HYGIENIC CONDITIONS IN VILLAGE SARINH, JULY, 1955



CONDITIONS	AREA 1 (POPULATION 115)	AREA 2 (POPULATION 239)
1. LOCATION	OUTSIDE VILLAGE	INSIDE VILLAGE
2. STREETS	STRAIGHT	NARROW, BAD SANITATION
3. HOUSES	MORE VENTILATED	SMALLER, DARKER
4. POPULATION	SPARSE	THICK
5. ECONOMIC STATUS	HIGHER (MILITARY)	LOWER (LABOR)
6. WELL	COVERED	UNCOVERED
7. WASTE WATER	DRAINAGE	UNDRAINED WASTE
8. ATMOSPHERE	DUSTY FROM ROAD	FAIRLY DUST-FREE

FIG. 5

FIGURE 6

CLINICAL STAGES OF EYE DISEASE BY AGE AMONG 1096 PUNJABI VILLAGERS IN SARINH, JULY, 1955

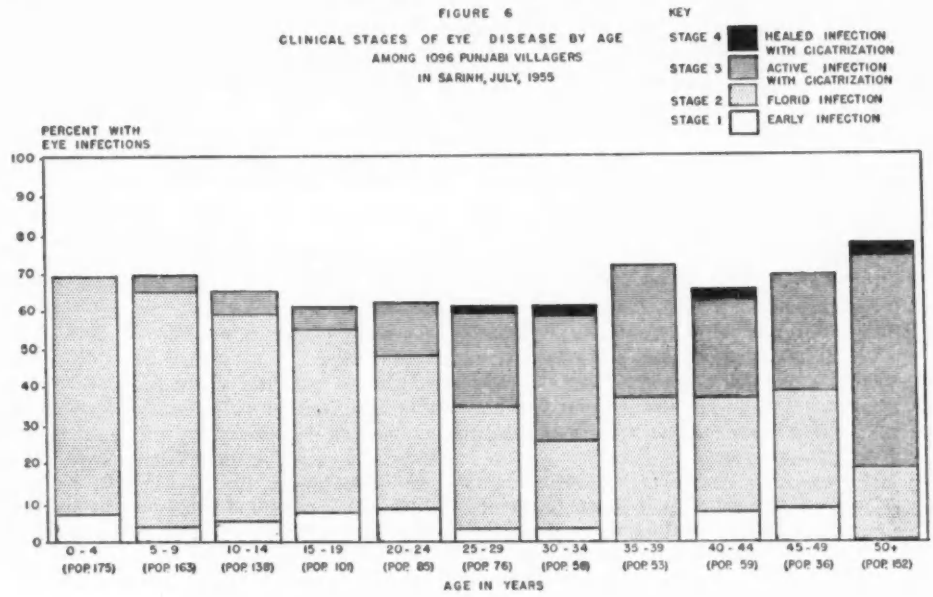


FIG. 6

workers who work as farm laborers, and the sweepers. Figure 3 shows the higher rates for low-caste people. Figure 4 shows the comparative sex distribution in the two caste groups and it is apparent that the male-female divergence is more marked in the upper caste groups.

Further data on the relationship of infection to socioeconomic status was provided by a more detailed study of two lower caste groups. The traditional home area of the leather-workers was inside the main village in an area where housing was crowded and sanitation unusually deficient, with a bad well, poor drainage and no open space (Area 2 in Figure 5). A group of men from the same caste group who had served in the military had settled in a separate community on the edge of the village (Area 1 in Figure 5). They had returned to the village to work and lived in the traditional way, but with accumulated military pay they had been able to provide themselves with better housing and other amenities. Calculating from the village map, the houses and courtyards in the new area outside the village provided about 400 square feet per person, while in the older area there were about 280 square feet per person. Eye infection rates were 69 per cent in the new area and 82 per cent in the older area, a difference which is significant with a P of less than .01 in populations of this size.

Stage of eye disease. Using the clinical criteria alone a sound impression of the stage of development of eye infections can be obtained although it is recognized that etiologic classification would contribute much more if it were possible. The stages we used follow the W.H.O. (MacCallan) classification of trachoma (W.H.O. Expert Committee, 1952) with the difference that we were unwilling to say how much of what we were seeing was a specific infection due to the unknown agent of trachoma. Stage 1 includes all cases of early conjunctivitis with only a few small follicles. About 14 per cent of children less than one year of age had Stage 1 infections with the overall rate ranging around 5 per cent (Figure 6). Stage 2 which includes florid conjunctivitis with well defined follicular and papillary hypertrophy forms the bulk of infections at early ages. Stage 3, which includes active infections with cicatrization, gradually takes over an increasing percentage of the population. Stage 4 which represents healing with visible cicatrization is relatively infrequent. The caste or sex distribution of the stages of infection did not show important variations from the above pattern.

DISCUSSION

In addition to the data presented there are certain epidemiologic observations which have come out of our field studies. They are worth noting even though quantitative proof cannot be presented.

It seems clear that the principle modes of transmission are direct and indirect contact. The phenomenon of reciprocating infection between mothers and children which we observed is well recognized in the epidemiology of most communicable diseases of childhood. There are many opportunities for direct contact with purulent discharges from infected eyes. Our observations suggest that the most important vehicle for the spread of eye infections in the Punjab is the village woman's "dopatta." This is a general purpose shawl which women wear around their shoulders or over their heads. It is used constantly for wiping the faces of children and the eyes of the mother as a substitute for washing. Another important source of spread is the "soormi" stick which is used to apply a black paste to the eyes of all members of the family. Although we do not have conclusive data to support these impressions they are based on prolonged and intimate observation of village health practices in an attempt qualitatively to evaluate factors important in transmission. The only way that we can see of obtaining quantitative epidemiological data would be to persuade a group of families to stop these practices and observe infection rates in their children. Flies may serve as vectors but probably are not as important as they seem to be in Egypt (Weir *et al.*, 1952).

Acute bacterial infections undoubtedly play an important part in the spread of the chronic infections generally labelled trachoma (Wilson, 1932). In school children with acute purulent conjunctivitis developing in previously normal eyes we have occasionally limited our treatment to a cleansing solution of boric acid or saline and have observed that as the acute infection cleared there was left behind a follicular conjunctivitis typical of what has been called Stage 2 trachoma. The large amount of pus discharged from severely inflamed eyes provides abundant transporting material for the postulated viral agents. Bacterial inflammation also may aid other agents in getting established by causing local irritation and damage (Poleff, 1951).

A feature of the physical environment which attracted our attention was the relationship between water and eye infections. In Transvaal (Amies *et al.*, 1953) and Japan (Mitsui, 1955) less trachoma was found in villages which had abundant water for washing than where water was limited. Surveys of children in the schools of four villages within 40 miles of Sarinh showed an interesting suggestive correlation between the number of ponds or "tanks" around the village and the eye infection rate. The villages with the most ponds had the largest infection rate. Villages without ponds have to rely on pumps and wells for bathing and washing, but there is no real deficiency of water. We have not yet been able to survey a sufficient number of villages to establish the validity of this observation which may be only a secondary association, but it is possible that quality of washing water will prove to be important.

Health education about the deceptively simple matter of cleanliness will in the long run probably be at least as important as treatment in control. There is no cultural resistance to the needed habit changes, but they can certainly be accelerated by better facilities such as a good water supply, soap and proper towels.

Our evidence suggests that in hyperendemic villages such as Sarinh treatment programs should most profitably focus on families. Our failure to control eye infections by working in schools alone is probably related to the amount of treatment given. Application of antibiotics three or four times a day for 1–2 month is the recommended course for complete cure. It was impossible to get our school teachers to carry out this ambitious program, especially since symptoms seemed to clear easily on much less treatment. We were able to get 30–40 per cent of the children completely free of clinical signs even with limited treatment. When treatment was stopped the rapid recurrence and clinical aggravation of eye infections was probably due both to relapse and to reinfection in the home. We turned to the family survey with the idea that a home control program though more difficult to administer would eventually be more effective. Many children, especially in the lower castes where eye infections are most prevalent, do not attend school and could not be reached by a school program.

Where a home control program is impractical, however, it may still be of some value to institute a control program based on schools. The progres-

sion of disease to severe late complications might be retarded. There would presumably also be some reduction in transmission not only in the schools, but also in the homes of school children. This is borne out by the higher prevalence of infection among mothers who are apparently being reinfected by their children.

SUMMARY

More than 90 per cent of school children in a Punjab village had eye infections. A control program with antibiotics applied once daily provided good temporary relief of symptoms and some cures, but infections rapidly returned when treatment was stopped.

Eye infections in the total population were then studied. An overall prevalence rate of 73 per cent was found. Almost half the children under one year of age were infected, rates rose to about 90 per cent during childhood and dropped to 60–70 per cent in adults. Women during the child-rearing age showed significantly higher infection rates than men. The relation to socioeconomic conditions is clearly shown by studies of caste groups, and there were important environmental features influencing spread of eye infections.

Control methods in Punjab villages should probably be focused on the homes where most of the spread of eye disease appears to occur. In addition to mass treatment, education to improve health habits is needed and would be aided by the provision of better facilities for keeping clean.

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OBSERVATIONS ON RESISTANCE TO SULFADIAZINE AND ANTIBIOTICS IN SHIGELLOSIS

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The intricate problem of adequate treatment of shigellosis appeared to have been solved with the advent of the sulfa drugs. Since 1941 reports have appeared stressing the specific activity of the various absorbable and nonabsorbable sulfa preparations in the treatment of this disease. At first the idea of administering a nontoxic, poorly absorbed compound with a high local concentration in the bowel promised to give the best and most consistent results. Thus sulfaguanidine and similarly acting drugs were and are still extensively used for the treatment of acute bacillary dysentery. On the other hand, absorbable sulfa drugs acted remarkably well on the more severe and toxic cases although their concentration in the intestine is low. The careful examinations of Hardy and his collaborators (1943; 1946) proved conclusively that the absorbable sulfa drugs are more efficient in their influence on the clinical course of the acute disease and in their sterilizing effect in the infected stools.

Thus, in general, treatment of shigellosis appeared to have been satisfactorily achieved in most countries, although occurrence of sulfa-resistant strains of *Shigella* became known in time. After introduction of the antibiotics in therapy, the various types of *Shigella* have been found to be sensitive to streptomycin, chloromycetin and the tetracyclines (Ross *et al.*, 1950; Mazursky *et al.*, 1950; McFadzean and Stewart, 1952; Abbott and Parry, 1955), but the favorable action of the sulfa preparations did not lead to the general use of antibiotics in the treatment of this disease. Apart from the observations of sulfa-resistant bacillary dysentery in American naval personnel on board ships in the Philippine Islands (Cheever, 1946), it was Tateno (1950) who referred for the first time to the predominance of sulfa-resistant bacillary dysentery in Japan in 1947 and confirmed these observations in additional studies (Tateno, 1951; 1955). Later on, Hardy *et al.* (1952), Zimmerman *et al.* (1952) and Garfinkel *et al.* (1953) reported on the high incidence of sulfa-resistant shigellosis in the large epidemics amongst prisoners of war during the Korean conflict. These groups of workers calculated 60 to 80 per cent of all cases observed to be sulfa-resistant, whereas therapy with the broad spectrum antibiotics, especially oxytetracycline and chloramphenicol, gave satisfactory results. In addition to these extended observations a number of reports have appeared dealing with the resistance of bacillary dysentery to sulfa drugs in the United States (Cooper and Keller, 1950), in England (Davies, 1954) and in the Belgian Congo (Verselder *et al.*, 1955).

In Israel bacillary dysentery is one of the endemic diseases frequently occurring in epidemic as well as in sporadic proportions. Incidence is high both in infants and in adults. Clinically the disease varies from the mild diarrheic forms to the fulminant dysenteric type, but is generally benign in character. Sulfa drugs were used here as early as 1939 and first clinical results of successful treatment were reported by Reitler and Marberg (1941). In the ensuing period the disease was successfully treated with the various sulfa compounds in accordance with the recommendations of the relevant literature. The first single cases of obvious sulfa-resistant shigellosis were observed by the senior author in 1951. They presented the picture of severe Shiga dysentery, were hospitalized, and subsequently successfully treated with chloromycetin.

With the more frequent appearance of similar resistant cases, it was decided to examine the problem in a larger series. Some aspects of these studies have been presented previously (Altmann *et al.*, 1956). Full results of these observations, carried out during the years 1953 till 1956, will be discussed in this report. They consist of combined bacteriological examinations and clinical evaluation in a number of patients suffering from bacillary dysentery, who were hospitalized from the central (mostly urban and surburban) parts of the country where shigellosis appears in single or massed endemic incidence. While the bacteriological examinations were carried out on isolated

TABLE 1

Shigella *species and serotypes studied*

		Series 1953–55		Series 1956	
Sh. dysenteriae	*1* (Shiga)	10		10	
	2 (Schmitz)	—		2	
	3 (Large-Sachs)	—		2	
	4 (Large-Sachs)	—		4	
	7 (Large-Sachs)	—		3	
Sh. flexneri	*1a*	5	67	12	71
	1b	—		2	
	2a	47		33	
	2b	1		—	
	3	11		3	
	4a	2		11	
	5	1		2	
	6	—		8	
Sh. boydii	*1*	—		1	
	2	—		1	
	4	—		1	
Sh. sonnei		23		5	
Total		100		100	

strains without prior knowledge of medical data, the clinical correlation had to be restricted to the more distinct and severe cases of the disease. Bacillary dysentery is one of those infections exhibiting in many cases mild and subclinical symptoms which render them unsuitable for clinical evaluation. Some cases were also excluded since for technical reasons sufficient clinical data were not obtained.

Two series of *Shigella* strains were examined bacteriologically. The first one was chosen at random out of several hundred cultures isolated during the period September 1953 to September 1955, but care was taken not to include strains from ward cross-infections. The second series comprises all *Shigella* strains isolated during the second half of 1956, those from cross-infections being again excluded as far as possible. The first series of examinations comprised 100 strains of *Shigella* isolated from 89 patients, while the second series of 100 strains came from 91 patients. The different species and serotypes are listed in Table 1, under international as well as conventional designation.

MATERIALS AND METHODS

All strains were kept on Stock Culture Agar (Difco) and examined in groups by the disk method and the tube dilution method. Sterile disks (6 mm. Whatman No. 1 filter paper) were saturated with the following solutions: sulfadiazine sodium (7,000 μg./ml.), chloramphenicol (2,500 μg./ml.) and tetracycline (2,500 μg./ml.). They were dried and kept dry at 4° C. Commercially available tablets were used for the estimation of sensitivity to neomycin. For the tube dilution method appropriate amounts of the above mentioned agents and of polymyxin B sulphate were added to fluid media to give final dilutions ranging from 0.1 μg./ml. to 500 μg./ml.

Peptone water (Tryptone) was the medium used for the tube dilution method and nutrient agar (BBL) for the disk method. Activity of sulfadiazine was tested on peptone-free synthetic media only. For most strains the medium of Kligler and Grossowicz (1941) was found satisfactory. More fastidious strains (especially *Sh. dysenteriae 1*) were tested on the more complex medium of Olitzki and Koch (1943) to which Uracil was added and nicotinamide used instead of nicotinic acid.

Inoculation was carried out from a 1:100 dilution with saline of an 18 hr. culture in brain heart infusion. The endpoint was considered to be the first tube showing no visible growth after 24 hours incubation. Tests on synthetic media were read after 24 to 48 hours. Strains growing poorly on synthetic media were not included in this investigation. Inhibition zones on plates were read after 24–48 hours and expressed as diameter in mm.

RESULTS OF SENSITIVITY TESTS

Sulfadiazine. Only the first series of strains (1953–55) was tested against sulfadiazine. All 10 *Sh. dysenteriae 1*, 32 out of 67 *Sh. flexneri* (49%) and 7 out of 23 *Sh. sonnei* (30%) were found resistant to sulfadiazine. These strains were not inhibited on plates, growing right up to the disk. In tubes, all but one grew in the presence of 500 μg./ml. No concentration higher than this was tried. The one outstanding culture was a strain of *Sh. sonnei* which grew in the presence of 70 μg./ml. For all practical purposes this strain must be regarded as resistant. Thirty five strains of *Sh. flexneri* and 16 strains of *Sh. sonnei* were found to be sensitive to sulfadiazine, being inhibited by 0.5 to 5 μg./ml. On plates, these strains did show zones of inhibition measuring at least 30 mm. Table 2 summarizes the results obtained by the tube method.

TABLE 2

Sensitivity of 100 strains of Shigella *to sulfadiazine on synthetic media. Series 1. (1953–1955)*

	Minimal inhibitory concentration of sulfadiazine in μg/ml						% of resistant strains
	0.5	1	2	5	100	>500	
Sh. dysenteriae 1	—	—	—	—	—	10	100%
Sh. flexneri 1a	2	—	2	—	—	1	
Sh. flexneri 2a	7	10	4	—	—	26	
Sh. flexneri 2b	—	—	—	—	—	1	
Sh. flexneri 3	2	3	3	—	—	3	49%
Sh. flexneri 4a	1	—	—	—	—	1	
Sh. flexneri 5	1	—	—	—	—	—	
Sh. sonnei	2	3	5	6	1	6	30%

TABLE 3

Sensitivity to chloramphenicol of 100 strains of Shigella *isolated 1953–1955*

	Minimal inhibitory concentration of chloramphenicol in μg/ml				Total no. of strains
	1	2	4	6	
Sh. dysenteriae 1	6	4	—	—	10
Sh. sonnei	5	9	8	1	23
Sh. flexneri 1a	4	1	—	—	
2a	33	14	—	—	
2b	—	1	—	—	67
3	10	1	—	—	
4a	1	1	—	—	
5	1	—	—	—	

Altogether one-half of all shigellae examined proved to be highly resistant to sulfadiazine. The clear-cut division between sensitive and resistant strains and the absence of intermediate ones seems remarkable. From 11 patients two or more strains were isolated on consecutive days. In 8 cases both isolates were sulfa-resistant. In one patient both isolates (*Sh. sonnei*) were sensitive. The cultures of the two remaining patients (both *Sh. flexneri 2a*) were at first sensitive, but strains isolated two days later proved to be completely resistant to sulfadiazine. Both patients were at first treated—unsuccessfully—with this drug.

Chloramphenicol. All 100 strains of the first series (1953–55) proved to be sensitive to chloramphenicol by both methods used. On plates, zones of inhibition were at least 25 mm. In tubes, *Sh. dysenteriae 1* and *Sh. flexneri* were inhibited by 1–2 μg./ml. chloramphenicol, *Sh. sonnei* being slightly less sensitive, as shown in Table 3. It was then concluded that all *Shigella* types were uniformly sensitive to chloramphenicol, which, therefore, could be highly recommended for treatment.

TABLE 4

Sensitivity of 100 strains of Shigella *to chloramphenicol and tetracycline. Series 2. (1956)*

	Minimal inhibitory concentration in μg/ml						
	Chloramphenicol				Tetracycline		
	5	75	100	100 ^	5	100	100 ^
Sh. flexneri	55	5	7	4	57	8	6
Sh. dysenteriae 1	7	3	0	0	7	2	1
Sh. dysenteriae 2–7	11	0	0	9	11	0	0
Sh. boydii	3	0	0	0	3	0	0
Sh. sonnei	5	0	0	0	5	0	0
Total	81	8	7	4	83	10	7

However, less than one year later, in 1956, we had reason to suspect the appearance of chloramphenicol-resistant strains. Out of the 100 cultures of the second series of strains examined, 16 *Sh. flexneri* and 3 *Sh. dysenteriae 1* were found to be resistant. On plates, these strains were not inhibited by disks. In tubes, 15 cultures grew in the presence of 50–75 μg./ml., while 4 were able to grow even in the presence of 100 μg./ml. chloramphenicol, the highest concentration tried in this experiment. The 16 resistant *Sh. flexneri* were isolated from 12 patients. In 3 cases the first isolates were sensitive, while strains isolated later proved to be resistant. Eighty-one cultures of this series were found to be sensitive to chloramphenicol. On plates, zones of inhibition of at least 25 mm. were observed. In tubes, they were inhibited by at least 5 μg./ml., the only low concentration tried in the examination of the second group. Results are summarized in Table 4.

Tetracycline. Eighty-three strains of the second series were found to be sensitive by both methods used. They were completely inhibited by at least 5 μg./ml., the lowest concentration tried. On plates, zones of inhibition were at least 25 mm. On the other hand, 14 *Sh. flexneri* and 3 *Sh. dysenteriae 1* were able to grow in the presence of 75 μg./ml. or 100 μg./ml. No higher concentration

was tried. On plates, these strains were not inhibited by disks containing this antibiotic substance, nor by disks containing chlortetracycline or oxytetracycline. An unexpected finding was that 16 out of the 17 resistant strains were the same as those found to be resistant to chloramphenicol. Only one tetracycline-resistant *Sh. flexneri* was sensitive to chloramphenicol, while out of 19 chloramphenicol-resistant strains only 3 were sensitive to tetracycline. Appearance of cross-resistance against different antimicrobial agents was considered to be mainly restricted to chemically related substances. But Kaipainen (1951), and Gocke *et al.* (1951) found that repeated exposure of different Enterobacteriaceae to chlortetracycline or oxytetracycline was followed by increased resistance to the homologous antibiotic as well as to chloramphenicol and vice versa, while sensitivity to neomycin remained unchanged.

Neomycin. All 100 strains of the second series were inhibited by at least 2 μg./ml. neomycin (base). On plates, zones of inhibition of 30 mm. average were observed.

Polymyxin. All 100 strains of the second series were inhibited by at least 0.5 μg./ml. of the drug, when tested by the tube method. No tests on plates were carried out.

CLINICAL EVALUATION

Correlation between the bacteriological examinations of the above two series of *Shigella* strains isolated during the years 1953–1956, and the results of clinical observations from a certain number of patients excreting these germs, gives the following picture.

In the first series, strains from 89 patients were examined bacteriologically. Forty-nine of these cases could be observed adequately and their reaction to treatment was evaluated. About 60 per cent of them were infants up to the age of 2 years, while 40 per cent were adults beyond 18 years. In a number of cases the dysenteric symptoms occurred before, together with or after febrile or chronic debilitating diseases.

The striking difference in the in vitro sensitivity to sulfadiazine as against that to chloramphenicol, as described above, is exemplified by these 49 patients. Whereas the strains from 23 cases were sensitive to the sulfa drug, the remaining 26 showed resistance. On the other hand all 49 strains were sensitive in vitro to chloramphenicol. The sulfa-sensitive group was composed of 21 Flexner and 2 Sonne strains, while the sulfa-resistant group consisted of 6 Shiga, 17 Flexner and 3 Sonne strains. The severity of the disease was in general more marked in the sulfa-resistant patients, while cases in the group sensitive to sulfa appeared to be less severe.

The amounts of sulfadiazine and chloramphenicol administered depended on the age of the patient. In adults about 4 grams of sulfadiazine per day was given, while 2 grams of chloramphenicol was used as a rule. Children received correspondingly smaller amounts. Treatment was continued on an average for three to six days. In addition patients received a bland soft diet, and in appropriate cases parenteral infusions with electrolytes and glucose were administered.

The results of treatment were assessed mainly according to the following signs: cessation of dysenteriform stools within two to four days, fall of temperature and other signs of general improvement. No attempt was made to obtain serial bacteriological examinations of feces during treatment, but random samples taken at the end of the diarrheic period usually failed to reveal significant numbers of positive isolations. The therapeutic results in this series of cases are summarized in Table 5.

As can be seen from these figures, 18 out of 23 sulfa-sensitive cases were treated with sulfadiazine and reacted favorably, while the remaining 5 patients received chloramphenicol, likewise with consistently good results. Twenty-two out of the group of 26 sulfa-resistant patients were initially treated with sulfadiazine, whereas 4 of them received chloramphenicol from the beginning. While all the latter 4 cases reacted promptly and favorably, the remaining cases, initially treated with sulfadiazine, showed varying results. Three of them recovered quickly in spite of their resistance to sulfadiazine in vitro. The causative agents were *Sh. flexneri* in 2 cases, and *Sh. sonnei* in the third one. Two cases, one caused by *Sh. dysenteriae 1* and one by *Sh. sonnei*, showed no response to treatment with chloramphenicol and recovered slowly. The remaining 17 patients, after the initial therapeutic failure with sulfadiazine, recovered promptly after treatment with chloramphenicol.

The series of 100 shigellae examined in 1956 contained, as mentioned before, strains from 15 patients, which were resistant in vitro to chlor-

TABLE 5

Response to treatment of 49 cases of bacillary dysentery. Series 1. (1953–1955)

Reaction in vitro	No. of cases	Types of clinical reaction after treatment with			
		Sulfadiazine	Chloromycetin	Sulfadiazine → Chloromycetin	Sulfadiazine → Chloromycetin
		Response +	Response +	Response − → +	Response − → −
Sulfa-sensitive	23	18	5		
Sulfa-resistant	26	3	4	17	2

amphenicol and tetracycline. Our interest was centered mainly on these cases and on the correlation between the bacteriological findings and the clinical course of the disease. One group of 6 patients (5 infants up to 1 year of age and 1 adult 75 years old) presented a severe course. The causative agents in 3 cases were *Sh. dysenteriae 1* and in 3 cases *Sh. flexneri*. Three of the patients died after a protracted illness showing signs of increasing toxemia. Treatment with sulfadiazine, chloramphenicol, tetracycline and, in one case, streptomycin, was ineffectual. Three other patients recovered only very gradually after having received sulfadiazine, chloramphenicol, tetracycline and in one case oxytetracycline. There was no indication that treatment significantly influenced the course of the disease. Two patients, 54 and 56 years of age, suffering from dysentery caused by *Sh. flexneri* and treated with chloramphenicol, recovered quickly, but on repeated cultures the causative organisms could be obtained during convalescence. The remaining group of seven cases (4 infants, 2 children, and 1 adult) with *Sh. flexneri* as causative agent, were clinically mild. One of them recovered without specific treatment, three received sulfadiazine, two chloramphenicol, and one tetracycline, but it was obvious that this therapy did not significantly influence the course of the disease, which was inherently benign. The clinical results of these 15 patients infected by strains of *Shigella* not sensitive to antibiotics are summarized in Table 6.

The above figures show that 40 per cent of the cases caused by antibiotic-resistant strains of *Shigella* were not significantly influenced by specific treatment. Three of these patients (half the number) died. While in 2 cases the resistance of the causative germs to antimicrobial agents was expressed only by the persistence of positive cultures after treatment, the remaining 7 cases recovered rapidly in contradiction to the results of the sensitivity tests, apparently due to the self-limited character of the disease.

TABLE 6

Response to treatment of 15 cases of bacillary dysentery. Series 2 (1956)

No. of cases	Per cent	Clinical course	Recovered	Died	Positive culture after treatment
6	40	Severe	3	3	
2	12	Mild	2		2
7	48	Mild	7		

DISCUSSION

Resistance to sulfa drugs in shigellosis is developing in Israel on lines parallel to developments indicated by observations previously made, mainly in Japan and Korea. The first clinical cases pointing to this trend were limited to infections with *Sh. dysenteriae 1*, a type which obviously has more clinical and epidemiological importance here than in other countries. The occurrence of individual differences in resistance to sulfa drugs in vitro is an oft observed phenomenon and *Sh. sonnei* especially has hitherto been notable in this respect. The development of resistance to sulfa during the course of the disease has likewise been described before, and our observation in two cases caused by *Sh. flexneri* reverting from sensitivity to complete resistance to sulfadiazine within two days, tends to confirm these findings.

In accordance with similar developments in other groups of pathogenic microorganisms, it is to be assumed that continued and extensive treatment has diminished the sensitivity of various types of *Shigella* to sulfadiazine to a varying degree. It is unclear, however, why this development has been conspicuous mainly in Japan,

Korea, the Philippines, and possibly Hongkong, while reports on this phenomenon from Europe and the United States are more sporadic.

The local trend during the last few years tends to be similar to the development observed in the Far East. In addition to our observation of an average of 50 per cent of sulfa-resistant *Shigella* strains, we have the examinations of Rozansky and Roman (1955), who found 80 per cent of another series of local *Sh. flexneri* to be resistant in vitro to sulfa compounds.

Our initial success in treating sulfa-resistant cases with chloramphenicol was soon followed by a decrease of antibiotic activity. About 15 per cent of 100 consecutively examined strains of freshly isolated shigellae proved to be insensitive to chloramphenicol and tetracycline in vitro. This lack of antibiotic activity was confirmed by a severe clinical course in 6 out of 15 cases of dysentery caused by insensitive strains, of which 3 died. This observation must be looked on with apprehension. Bacillary dysentery, after having been almost deleted as a problem with important clinical implications, may revert to its former clinical severity, thus demanding again new means of therapy.

From a practical point of view the choice of an active antimicrobial agent in cases of bacillary dysentery thus becomes a question of major importance. Early isolation of the causative organism with sensitivity tests in vitro may be necessary to allow the successful choice of an antibiotic agent in those cases not reacting to routine treatment. The obvious sensitivity in vitro of *Shigella* to polymyxin and neomycin observed in our studies has not yet been sufficiently followed up by therapeutic assessment. The experience with these drugs (Ross *et al.*, 1949; Rogers *et al.*, 1956) leaves doubt as to their efficacy and compatibility, but future developments may necessitate their being used together with other suitable agents if the trend toward acquiring resistance to antibiotics by *Shigella* continues.

SUMMARY

Combined bacteriological and clinical studies were undertaken to determine the sensitivity of *Shigella* to sulfadiazine and antibiotics. About 50 per cent of the strains of *Shigella* examined were resistant to sulfadiazine in vitro, and a similar lack of response to treatment with this drug was observed in cases caused by resistant strains. While chloramphenicol was the drug of choice in sulfa-resistant shigellosis until 1955, strains were isolated in 1956 which proved to be resistant to chloramphenicol and the tetracyclines. A number of cases of acute shigellosis caused by such resistant strains took a clinically severe course and some of them died.

The need for planned treatment of bacillary dysentery is indicated by the above findings, and the introduction of drugs like neomycin or polymyxin, which were found to be very effective in vitro in the treatment of cases caused by resistant strains, must be taken into consideration.

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RESEARCH ON SCHISTOSOMIASIS JAPONICA IN CHINA

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Extensive research on schistosomiasis was made in China in 1956 with the National Research Council on Schistosomiasis spearheading the project. During the second session of the Council held from December 4 to 12, 1956, more than 200 papers were presented, the essentials of which are as follows:

EPIDEMIOLOGICAL STUDIES

The year 1956 was an important one for it was the first of the designated seven to twelve years in which schistosomiasis is to be liquidated in the country. It was also the year when all technical, material and personnel preparations had to be made for the huge undertaking.

Armed with the general knowledge on schistosomiasis, villagers were able to report the occurrence of the intermediate host, *Oncomelania* snails, and/or the existence of patients with visible symptoms such as ascites. Guided by such information, the staffs of local anti-schistosomiasis stations were able to start a systematic survey on the distribution of *Oncomelania* snails and the incidence among the population. Infection was diagnosed chiefly with the intradermal reaction, seconded, when necessary, by stool examination consisting of both the hatching technique and sedimentation method. Altogether 58,000,000 intradermal tests were undertaken last year. As a result, more accurate data concerning the distribution and the incidence of the disease were gathered: one hundred million people were estimated to be under its constant threat. The endemic area covers eleven provinces and the Municipality of Shanghai. This is an area four times the size of France or nine times that of the United Kingdom. The eleven provinces, mostly south of the Yangtze River, are Kiangsu, Chekiang, Anhwei, Fukien, Kiangsi, Kwangtung, Kwangsi, Hunan, Hupeh, Yunnan and Szechwan.

As to the reservoir hosts, besides cattle, buffaloes, milk cows, sheep, goats, pigs and horses which had already been proved in the past to harbor schistosomes under natural conditions, several species of wild animals such as *Felis bengalensis chinesis* Gray, *Mustela sibirica davidina* Milne-Edwards and badgers (*Meles meles*) were also incriminated. In one of the villages in Mienchu County, Szechwan Province, the incidence among cattle was as high as 91 per cent. Liver-puncture has been proved to be a valuable diagnostic method in detecting schistosomiasis of sheep.

The discovery of *Oncomelania* snails in coastal regions high in salinity and alkalinity refuted the assertion that oncomelaniid snails breed only in fresh-water. Observations throughout the year on the reproductive organs of male and female snails in the Chinkiang area (Kiangsu Province) revealed that similar to studies made in other parts of China, there does exist a cyclic development. Snail food was found to consist of a great variety of plants: Graminae, Bacillariophyceae and Polypodiaceae. Direct observation proved that the role of radula is to catch food particles into the buccal cavity without playing any part in the action of mastication. By pumping dry a section of an infested stream or rivulet so as to facilitate direct counting of snails above and below the water level, it was found that *Oncomelania* snails do exist in water at any time of the year. The presumption that snails live in water during the summer and out of water during the winter should be revised.

PREVENTIVE STUDIES

Extermination of snail host. It has been demonstrated by the studies in the past few years that snails when buried to a depth of more than 10 cm. succumbed within 3–6 months. Consequently infested irrigation canals were treated that way by the farmers under the technical supervision of anti-schistosomiasis stations. During the year 1956, extermination of snails by this method was initiated over an area of 158,400,000 m². As to snails scattered in vast swamps and lowlands, which were considered as very difficult, if not impossible, to eradicate, it has been proved by

field experiments that extermination is possible when coupled with agricultural utilization of the land by draining and deep ploughing.

Calcium arsenite, sodium pentachlorophenate, benzene hexachloride, alcoholic extract of *Croton tiglium* and calcium cyanamide were the few molluscicides studied last year. Calcium arsenate had been tried as a new molluscicide by Chinese authors, but recently an analogous compound, calcium arsenite, was found to possess the same molluscicidal property, yet was much cheaper and easier to manufacture than the former. Satisfactory results are obtained with the spraying of 500 ml. of 1% suspension per m^2.

The use of torches for snail-killing was also a new device introduced last year. Gas generated from the slightly modified boiler of an ordinary small steamer was able to produce a flame 0.5 m. long, killing more than 90 per cent of the snails in the area under treatment.

In the field of biological control with natural enemies, pathogenic micro-organisms as well as parasitic nematodes have been studied. Besides, certain species of fresh-water crabs have also been found to be predators; one crab was seen to consume more than a hundred snail specimens at one time.

Fecal control. Chinese authors demonstrated in 1952 that the death of schistosome eggs in stored night-soil was due to the toxic action of ammonia rather than the heat produced from fermentation. When fecal materials were stored with an adequate proportion of urine, the death of schistosome eggs was greatly hastened. In 1956, it was further demonstrated that the addition of calcium cyanamide, which itself is a useful fertilizer in the concentration of 0.3%, would ensure not only the death of schistosome eggs but also that of hookworm eggs. Heat produced by the composting method is sufficient to kill the eggs of the blood fluke and the method is applicable to human as well as to animal excreta.

Cercaricidal studies. When Halazone was used to disinfect water with residual chlorine of 1 p.p.m., schistosome cercariae were killed within 15 minutes at 13–15°C. or within 5 minutes at 25–28°C. Similar results were obtained with high-test calcium hypochlorite. When infested water was treated with tincture of iodine at a concentration of 8 p.p.m., cercariae were killed within 5–7 minutes at 13–15°C. or within 3–4 minutes at 25–28°C.

FIG. 1. Workers from the Wusi Anti-Schistosomiasis Station in East China treating an infested irrigation canal with 666.

Fine cloth impregnated with 10% benzyl benzoate retained its protective property against penetration of schistosome cercariae for 9 hours. Two layers of fabric with powder of *Camellia oleosa* in between afforded the same protection. The absence of cercariae in deep water was proved by exposing laboratory animals to infested water. For practical purposes, the blind end of a water course was separated from the main body with a dam, snails within the section were thoroughly exterminated and night-soil receptacles were removed far away from the banks, after which water was allowed to flow back to that section through a tunnel under the dam and was found to be practically safe for domestic use.

CLINICAL AND THERAPEUTIC STUDIES

In 1956, the effectiveness and side-effects of tartar emetic following 3 and 7-day intensive treatments were clinically compared. Of 6,050 cases treated with the 3-day course, 1,220 were followed up for 3–6 months with a relapse rate of 29.1 per cent; of 3,834 cases treated with the 7-day course, 426 have been followed up with a relapse rate of 22.1 per cent. Toxic effects in the 7-day schedule were somewhat more frequent and severe in nature than those in the 3-day one.

FIG. 2. At the Soochow No. 2 People's Hospital. Western-trained doctors discussing a case with practitioners of traditional Chinese medicine.

Earlier experiments with laboratory animals had shown that procaine could enhance the efficacy and at the same time alleviate the toxicity of tartar emetic; but the results of trials in 400 clinical cases in 1956 were rather disappointing, irrespective of whether procaine was used parenterally or by injection, before or after the administration of tartar emetic. The effect of glucose in high concentration in reducing the toxicity of potassium antimony tartrate was still a controversial subject among clinicians. Though more than 2,000 clinical cases have been observed, opinions were far from unanimous.

Both clinical observation and laboratory experimentation have proved that in most cases, fatalities following antimony treatment are due to cardiac disorder, especially ventricular fibrillation, attributable to intoxication of cerebral cells with antimony; as a result, the excitability of the central nervous system is unbalanced, causing an exaltation of excitability of the vagus and the myocardium. Consequently, the use of atropine in big doses would be very helpful in a certain number of intoxicated cases.

The examination of autopsy materials and surgical specimens revealed that schistosome eggs were found in the large intestines, liver, small intestines, appendix, mesenteric lymph nodes, lungs, spleen, pancreas, stomach, heart, inguinal lymph nodes, cervix of uterus, fallopian tubes, psoas muscle, post-mesenteric lymph nodes, omentum and epiploon.

Among 1,049 schistosomiasis cases recorded in a hospital in Shanghai during the past 5 years, 45, or 4.29 per cent showed nervous symptoms. Adult worms have not been demonstrated in histological section of the brain. Thickening of the meninges, adhesion, exudation, small tubercles, granulomata containing schistosome eggs and cerebral edema were the lesions usually encountered. Other ectopic or rare lesions were: dermal pustula containing eggs, constrictive pericarditis due to the presence of eggs, intestinal occlusion, endocardial obstruction, fistula of the sigmoid and intestinal intussusception.

In the field of diagnosis, in addition to the successful use of adult worm antigen for intradermal tests elaborated in the past few years, antigens prepared from liver tissue containing huge number of schistosome eggs or prepared directly from

purified miracidia were studied as to their efficacy and practicability. Besides, the complement-fixation test, precipitation test (ring test) and CHR have also been studied with respect to their sensitiveness and specificity. The last two serological tests have been found to be useful methods in early diagnosis of the disease. In experimental schistosomiasis of rabbits, the sedimentation of red blood corpuscles was accelerated and the acceleration was correlated with the intensity of the infection.

PHARMACOLOGICAL STUDIES

More than a hundred new chemical compounds were synthesized and tested for their schistosomicidal effect during the year 1956. Among them, Sb-5 ($C_6O_5H_8S_2KSb$), Sb-11 ($C_8O_5H_8NaSb$) and ammonium antimony gluconate showed a stronger schistosomicidal action than tartar emetic. Studies on sodium antimony (pentavalent) gluconate proved that its toxicity and efficacy varied with changes in the manufacturing process, especially in the change of pH. It was proved furthermore that the toxicity and the efficacy of the compound did not run in parallel. Among non-antimonial compounds, besides several analogous to symmetrical diaminodiphenoxyheptanes which, in experimental therapeusis, were not so effective as reported by British authors, 2 tin preparations and 2 out of 24 derivatives of Miracil D have proved to possess a certain schistosomicidal action in experimental schistosomiasis of mice.

Experimental therapeutic studies indicated that the effectiveness of antimonial compounds was not necessarily correlated with their antimony content. Even for a given compound, for example ammonium antimony gluconate, beyond a certain limit the increase of antimony content was not accompanied by an increase in its therapeutic value or in its toxicity.

By studying conditioned reflexes and summation of impulses in animals treated with tartar emetic, it was demonstrated that antimony could produce inhibitory action on the central nervous system, including the cortex. Perfusion of the internal receptors, compression of the carotid artery and excitation of both ends of the vagus, etc., proved that the stimulation of receptors did increase the excitability of the vagus and the sympathic nerves, thus predisposing to the appearance of ventricular fibrillation.

In experimentally infected dogs suffering from acute intoxication by tartar emetic, the liver glycogen was markedly decreased or even completely eliminated. Blood sugar was momentarily slightly increased, followed by a decrease to convulsion level. Blood calcium was not markedly changed. Hypoglycemia and hypoglycemic convulsion led finally to respiratory paralysis resulting in the death of the animal. The assumption by foreign authors that death occurs as a result of shock was disproved. The glucose content of adult schistosomes was greatly reduced within half an hour after the injection of tartar emetic into the host animal, but it was restored within 12–48 hours and even surpassed the normal value one month after.

Since some enzymes involved in the metabolism of glucose are proteins containing an active SH group and since antimony can hinder the enzymatic activity by combining with the SH group, chemical compounds containing the SH group were tried as disintoxicating agents. Of these, disodium dimercaptosuccinate was found to possess a powerful disintoxicating action towards antimonials. When treated with this compound, mice were able to tolerate tartar emetic at 15 times the LD_{50}, but at the same time antimony was also rendered ineffectual. The antimony content in the female schistosomes was twice that in the males and was richer in the liver than in other internal organs. The administration of di-sodium dimercaptosuccinate markedly lowered the antimony content in the female schistosomes and the liver tissue.

Anti-thyroid drugs have been proved to possess a disintoxicating effect towards antimonials in laboratory animals.

Among suppressives, chloral hydrate was found able to reduce the toxicity of tartar emetic without affecting its effectiveness in experimental schistosomiasis of mice; but bromides and barbitals behaved quite differently, as toxicity was increased by their use. In experimentally infected rabbits, it was proved that the occurrence of fever during antimony treatment would increase the toxicity and the effectiveness of tartar emetic as well; while the use of pyramidon during febrile attacks would decrease the schistosomicidal effect without alleviating the increased toxicity.

Many improvements have been made in methods of experimental therapeusis. Since antimonials were able to cause an hepatic shift of schistosomes in animals under treatment, a quick method for screening new drugs was devised which consisted in administering a single maximum-tolerated dose and examining after two hours the percentage of worms shifted to the liver as well as that of unpaired worms. Some useful hints about the therapeutic value of the drug under test were thus obtained with much less labor and time.

During the past few years, studies have been undertaken on the cultivation of schistosomes. Improvements in the culture flasks and media resulted not only in the prolongation of the survival of schistosomes *in vitro* but also in a slight development of the worms. In 1956, studies were made with 24 amino-acids as to their effect on the survival of blood flukes *in vitro*. It was found that most amino-acids acted favorably on their survival, especially the prolines which increased the survival of schistosomes by 5.7 times as compared with the controls.

The chemo-prophylactic property of rosaniline dyes was ascertained by animal experimentation.

Space does not allow us to enumerate all the achievements of the past year nor to go into details of any particular subject which must be reserved for special reference of publication. Suffice it to point out that general planning under a unified leadership and the full cooperation of the Ministry of Agriculture, Ministry of Health and Ministry of Water Conservancy in the anti-schistosomiasis campaign provided a strong guarantee of the success of research work in the prevention and treatment of the disease. The aim of scientific research is to serve the anti-schistosomiasis campaign and all the useful results obtained such as the use of intradermal reaction in the epidemiological survey, the deep-burying of the snail host and the extensive trial of the 3-day treatment were immediately endorsed by the administrative authorities and executed in all the endemic areas. The intimate correlation between mass campaigns and technical application is a very outstanding feature in scientific work in China today.

A PRECIPITATION REACTION FOR THE DIAGNOSIS OF CYSTICERCOSIS

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Pork infected with *Cysticercus cellulosae* is frequently reported in slaughter houses in Mexico (Mazzotti, 1954) and human infections with *Taenia solium* are not rare, giving rise to cases of cysticercosis which in Mexico City is second only to amebiasis as a cause of human mortality due to parasitosis. In 1,339 autopsies, predominantly adults, made over a period of 4 years at the Pathology Unit of the National School of Medicine there have been 39 cases of cysticercosis.

This infection is favored by bad habits in personal hygiene and food handling as much as by poor disposal of human excreta. Its clinical importance and manifestations depend on the localization and the number of parasites. Cerebral cysticercosis is the most dangerous, but ocular, muscular, subcutaneous and cardiac localizations may be serious. While it is possible for an experienced person to diagnose cysticercosis, it is usually established only after surgical intervention or autopsy. At present the complement-fixation reaction and the precipitation reaction (PR) with *Cysticercus* antigen are the only serologic aids in diagnosis. The former was introduced by Weinberg in 1909 (cited by Huhn, 1956). With spinal fluid this reaction is sensitive and usually specific, but gives false positives in cases of neurosyphilis and in general gives numerous false positives with serum (Nieto, 1948; Vosnaya, 1953). Rothfeld (1935) used PR with good results in 4 human cases of cysticercosis but he did not evaluate his antigen and nothing further has been reported. Since the latter technique is simple and economical the present investigation was undertaken.

MATERIAL AND METHODS

Fresh *Cysticercus cellulosae* was obtained from pigs, and 22 antigens were prepared from the whole *Cysticercus*, the vesicular fluid, scolex and membrane by the following methods.

1. The helminth was crushed, fat removed with petroleum ether (Ligroine), extracted in Evans' solution[1] at 5°C., brought to pH 7 and sterilized by Seitz filtration.

2. Helminth was crushed and lyophilized.

3. Crushed and washed 2 or 3 times with acetone for 15 minutes, dried at room temperature (not more than 2 hours) and pulverized in a mortar.

4. The same as #3 with extraction in Evans' solution[1] at 5°C. for 3 days, dialysed through cellophane at 5°C., brought to pH 7 and sterilized by Seitz filtration.

5. Crushed in methyl alcohol, allowed to stand 15 days at 20°C., filtered and the solvent evaporated.

6. Fat removed with petroleum ether (Ligroine) and lyophilized.

The powdered antigens were suspended in a 0.85% solution of NaCl, 1:100; maintained at 37°C. for 1 hour and at 5°C. for 24 hours. The powdered and dissolved antigens were refrigerated at −20°C.

Porcine sera with or without cysticercosis as verified by *post mortem* examination, human spinal fluid and sera selected at random from persons of all ages, as well as spinal fluid of verified human cases of cysticercosis were obtained.

The PR were carried out in test tubes of 6 mm. inner diameter, introducing first 0.1 ml. of serum or spinal fluid to form an interphase with 0.1 ml. of antigen in dilutions of 1:100, 1:500, 1:1,000, 1:2,000, 1:5,000, 1:8,000, 1:10,000 and 1:12,000. The tubes were incubated at 37°C. for 1 hour and at 5°C. during 3 hours. The reaction was considered positive when a white ring formed at the interphase.

RESULTS AND DISCUSSION

Of the 22 antigens 9 yielded false positives with negative control sera and were discarded. Four of the antigens were positive with high dilutions of known positive sera (Table 1). In decreasing order of sensibility these antigens were:

[1] Evans' solution: NaCl 50 g.; KH_2PO_4 3.63 g.; Na_2HPO_4 14.31 g.; H_2O 1,000 ml.

TABLE 1

Precipitation reaction with various antigens and sera of pigs with cysticercosis

Method		1	2	3	6
Antigen		A B C	E F G	H J K	S T U V
Serum	1:1,000	+++	+++	+++	++++
	1:2,000	+++	+++	+++	++++
	1:5,000	+−+	+++	+++	−+++
No. 9	1:8,000	+−−	−+−	+++	−−−−
	1:12,000	−−−	−−−	−−−	−−−−
Serum	1:1,000	+−−	−++	+++	+−+−
	1:2,000	+−−	−++	+++	+−+−
	1:5,000	−−−	−−−	++−	−−−−
No. 10	1:8,000	−−−	−−−	++−	−−−−
	1:12,000	−−−	−−−	−−−	−−−−
Serum	1:1,000	−−−	−+−	+++	−+−−
	1:2,000	−−−	−−−	−+−	−+−−
	1:5,000	−−−	−−−	−−−	−−−−
No. 11	1:8,000	−−−	−−−	−−−	−−−−
	1:12,000	−−−	−−−	−−−	−−−−

TABLE 2

The number of positive sera at different dilutions in PR with Cysticercus cellulosae *antigen (H)*

	1:2,000	1:5,000	1:8,000	1:12,000
Porcine sera with cysticercosis	0	5	4	2
Positive human sera	1	1	1	3

TABLE 3

PR with Cysticercus cellulosae *antigen, 1:100, and 1:2,000*

Sera	Total of samples	Total positives	Positive percentage
Porcine, with cysticercosis	39	39	100
Porcine, normal	50	1	2
Human, unselected	1108	36	3.2
Human spinal fluid			
Unselected	1043	1	0.1
Cysticercosis *cellulosae*	3	3	100
Cysticercosis *racemosus*	6	1	16.7

Table 4

PR with Cysticercus cellulosae *antigen in different parasitoses*

Number of sera	Parasite	Positive PR
1	*Leishmania tropica*	0
5	*Entamoeba histolytica*	0
4	*Entamoeba coli*	0
5	*Fasciola hepatica*	0
10	*Taenia sp.*	0
5	*Hymenolepis nana*	0
7	*Ascaris lumbricoides*	0
8	Hookworm	0
4	*Strongyloides stercoralis*	0
9	*Trichuris trichiura*	1
7	*Mansonella ozzardi*	0
5	*Cysticercus pisiformis*	0

TABLE 5

PR with human spinal fluid and Cysticercus racemosus *antigen (1:100, and 1:2,000)*

	Total of samples	Total positives	Positive percentage
Unselected	7	0	0
Cysticercosis *racemosus*	3	3	100
Cysticercosis *cellulosae*	1	0	0

Antigen J: membrane—method 3
Antigen H: whole *Cysticercus*—method 3
Antigen K: scolex—method 3
Antigen F: membrane—method 2

As antigen H is almost as sensitive as J and does not require dissection of the cysticerci in its preparation, it was selected for tests the results of which are presented in Tables 2 and 3.

The contrasting results of the reactions with sera from infected and uninfected pigs are noteworthy (Tables 2 and 3). The number of positive PR in unselected human sera is very low and even if these were not due to cysticercosis, 3.2 per cent is an acceptable margin of error in a serological reaction. The clinical histories of 6 children in this series with a positive PR showed only one with convulsive attacks suggestive of a cerebral cysticercosis. The one positive spinal fluid from 1,043 unselected human sources (Table 3) belonged to a child with a history of convulsions, vomiting and blindness, clinically compatible with cerebral cysticercosis.

Among the PR negative spinal fluids there were 13 patients with neurosyphilis and a positive Wasserman test in the spinal fluid, 50 patients with diverse, nonparasitic diseases of the central nervous system, 2 patients with taeniasis and 3 others with intracranial calcifications, similar to those produced by healed, calcified *Cysticercus*.

Positive reactions were obtained in all three patients tested so far with cysticercosis *cellulosae*, whereas only one of six patients with cysticercosis *racemosus* was positive. Only cases verified personally after surgery or autopsy have been considered, and since we had the material we prepared antigen from *Cysticercus racemosus* obtained surgically and performed PR according to Table 5 with antigen prepared following Method 3. The number of spinal fluids so far tested with this antigen is small but it may be noticed that the reaction was positive in all cases of cysticercosis *racemosus*. It seems that antigens from *Cysticercus cellulosae* and *C. racemosus* are specific for their respective types of cysticercosis. So far, only one case of cysticercosis *racemosus* has been positive with antigen from *C. cellulosae* at a dilution of 1:2,000; the same case, however, was positive with antigen from *C. racemosus* at a dilution of 1:12,000.

Table 4 presents the results of the reactions of *cellulosae* antigen with sera derived from persons with various other parasitoses including taeniasis, as well as from 5 rabbits infected with *Cysticercus pisiformis*. With one exception the tests were uniformly negative.

With these results we believe that the PR with *Cysticercus* antigen (H) in both serum and spinal fluid gives great promise in the diagnosis of cysticercosis. We hope to evaluate it further in, proven cases of human cysticercosis.

The dissolved antigen or its powdered form remained active after more than 5 months at −20°C. and for more than 7 days at 5°C. The positive and negative sera retained their activity after undergoing temperatures of 56°C. for 30′ 5 days at room temperature, 7 days at 5°C. and several months at −20°C., but upon bacterial contamination the serum and spinal fluid became inactive.

Of primary importance is the careful separation of the cysticerci from the pork meat and fat as well as its total desiccation after acetone treatment.

SUMMARY

One of 22 prepared antigens of *Cysticercus cellulosae* was selected and tested with the sera of 39 pigs with cysticercosis, 50 normal pigs, 1108 unselected human sera, 1043 unselected spinal fluids and 9 spinal fluids from human cases of cysticercosis. The reaction was sensitive; it did not give cross reactions in various parasitoses including taeniasis, or in neurosyphilis. Antigen from *Cysticercus cellulosae* gave a positive reaction in all tested cases of cysticercosis *cellulosae*, and antigen from *C. racemosus* in all tested cases of cysticercosis *racemosus*. It will be useful to continue its evaluation in proven cases of human cysticercosis.

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EFFECTS OF PHTHALOFYNE[1] IN THE TREATMENT OF HUMAN *TRICHURIS TRICHIURA* INFECTION

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The prevalence of the intestinal parasite of man, the whipworm *Trichuris trichiura*, and the inadequacies of the several modes of treatment now in use are generally well appreciated. The pathogenicity of this worm when present in large numbers, particularly in infants, is well recognized. Recently the death of a 5-year-old child heavily infected with this parasite and without autopsy evidence of other cause of death was reported by Gajardo and Atias (1956). It may well be that even moderate or small numbers of whipworms in adults are not so innocuous as was formerly believed.

The clinical situation with respect to the whipworm *Trichuris vulpis* in dogs was very similar. Fortunately, the drug phthalofyne (3-methyl-1-pentyn-3-yl acid phthalate) (Whipcide) has been found to be very effective as single dose therapy in ridding dogs of this parasite, and apparently this drug is safe enough for general use in dogs, as indicated in reports of Burch (1954), Ehrenford *et al.* (1955), and Bunde (Personal communication, 1956). Hoekenga (1956), noting the effectiveness of this drug in dogs, has reported on its use in human beings infected with *Trichuris trichiura*.

Hoekenga treated 36 adult Honduran mestizos with doses of phthalofyne ranging from 20 to 145 mg./kg. of body weight, per day for 4 or 5 days, attaining a 22 per cent cure rate in this group. Sixty fasting patients received a single dose of from 100 to 200 mg./kg., and 47 received similar doses approximately 2 hours after a meal. He reported an over-all cure rate of about 50 per cent in this group of 107 patients, with slightly better results in the group that took the drug postprandially. Approximately 9.5 per cent of these 107 patients developed conjunctivitis and keratitis about 12 hours after having taken the drug. These complications were noted equally in the fasting and the nonfasting groups, and gradually subsided in 7 or 8 days. Seven additional patients received more than 200 mg./kg. of body weight, and all 7 developed conjunctivitis and keratitis. In some severe cases inflammation did not subside for 3 weeks. Two of these patients developed moderate deafness.

In the dog, toxic manifestations have appeared only after much larger doses than those necessary to eradicate the dog whipworm, and careful examination has revealed no ocular toxicity (Bunde, personal communication, 1956). Chimpanzees are said to have developed signs of ocular toxicity similar to those noted in human beings.

The present study was undertaken with the hope of developing a therapeutic regimen with phthalofyne which would be both effective and nontoxic.

METHODS

Ten adult male Filipinos between 22 and 25 years of age, who had been born and raised in Cavite Province, Philippines, and who were currently residing in the Seattle area, cooperated with us in this study. These men denied physical complaints and were found to be physically fit. All were of average height and weight for their race, and each had been receiving a generally adequate diet. Laboratory studies, including leukocyte count, hemoglobin level, erythrocyte sedimentation rate, urinalysis, and chest roentgenogram, were normal in all patients, and their serodiagnostic tests for syphilis were negative. Each was noted to have a slight eosinophilia ranging from 6 to 13 per cent. The majority of these patients had been previously treated for other intestinal parasites prior to this study. Nine of the 10 had received previous treatment with other drugs at least one month prior to the present study, and the remaining patient had received tetrachlorethylene without apparent toxic effects one week prior to treatment with phthalofyne.

The patients were hospitalized under close

[1] Phthalofyne (Whipcide) and phthalofyne acid (P-403A) produced by Pitman-Moore Company, Indianapolis, Indiana, were kindly furnished for this study through the courtesy of Dr. Carl A. Bunde.

observation for the period of preliminary study, administration of phthalofyne, and for at least one week thereafter. At least 1 pretreatment and 3 post-treatment stool specimens were carefully identified, examined with concentration methods, and the findings checked by a competent microbiologist.

Phthalofyne was administered personally by a physician with avoidance of unduly alarming the patient, and dosage and retention of the drug were verified. The drug was given in the form of candy-coated, capsule-shaped tablets, each containing 0.5 gm. of phthalofyne. (By placing a few tablets in tap water it had previously been confirmed that they disintegrated to powder readily and it was therefore assumed that such disintegration would readily occur in people.) The dosages given involved the swallowing of from 11 to 22 of these 8 by 19 mm. tablets, taken with a little water or milk. Each patient took all his tablets in an unhurried fashion at one sitting.

A specialist in ophthalmology and otolaryngology carefully examined the eyes and ears of each patient on the day of drug administration and for several days thereafter.

The sodium salt of phthalofyne, given as an enema solution to Cases Nos. 9 and 10, was prepared by adding half the quantity, by weight, of sodium bicarbonate to the measured amount of phthalofyne acid (P-403A, Pitman-Moore Company) in enough distilled water to make the final desired concentration. This was stirred and allowed to stand until foaming ceased. The resulting solution produced no irritation when applied to the buccal mucosa.

CASE REPORTS

Case No. 1 was given 100 mg. of phthalofyne per kilogram of body weight about 1½ hours after breakfast. No toxic effects of the drug were found, and the number of *Trichuris* eggs noted on stool examinations performed repeatedly for one week after therapy was essentially unchanged as compared with the pretreatment estimate of 50 to 72 eggs per gram of feces.

Cases Nos. 2 through 5 received 125 mg. of phthalofyne per kilogram 1½ hours after breakfast. Each of these patients independently stated that he noted slight nausea before lunch. All were able to eat at least a part of their lunches, but Case No. 5 vomited or regurgitated a portion of the food eaten at that meal. All were asymptomatic by supper time, and no other untoward reactions were found. There was no evidence of significant reduction in the number of whipworm eggs noted in stools collected during the week following therapy. Stool specimens from cases Nos. 2 and 5 continued to contain normal-appearing *Trichuris* eggs 4 months after therapy.

Cases Nos. 6 and 7 were given a castor oil purge one evening and a tap water enema the following morning. They were allowed to eat breakfast and then were given 125 mg./kg. of phthalofyne 1½ hours later, and were given an equal amount of phthalofyne suspended in 1,000 ml. of water containing a small amount of barium sulfate as an enema, followed by 1,000 ml. of stock barium sulfate enema solution. By fluoroscopy the enema suspension appeared to reach well into the ileum, and no abnormalities of the terminal ileum or colon were found. Each patient retained the enema for ½ hour before expelling the material. These patients exhibited no untoward effects attributable to the phthalofyne and again there was little if any change in the numbers of whipworm eggs found in the stools examined following therapy. Case No. 7 was reexamined five months after therapy, and *Trichuris* eggs were demonstrable at that time.

Case No. 8, like most of the others, had received other forms of therapy prior to the present study, but *Trichuris* eggs persisted in his stools. He was given 175 mg. of phthalofyne per kilogram of body weight by mouth after breakfast. Shortly after having eaten his lunch, he vomited apparently all of his gastric contents. His stools also continued to remain positive for *Trichuris* eggs. It is to be noted that this was the largest oral dose of phthalofyne given to any of the patients in the study and it was insufficient to eradicate infection. The occurrence of pronounced nausea and vomiting in this case would suggest that even though higher oral doses might be trichuricidal, they would not be well tolerated and very likely not retained by the patient.

Case No. 9 received no oral phthalofyne. He was given 2 enemas, a week apart, consisting of a suspension of the sodium salt of phthalofyne. On the first occasion the enema solution was made from 11 gm. of phthalofyne acid (200 mg./kg.) in 150 ml. for a 7½ per cent solution, and on the second occasion, 25 gm. of phthalofyne acid (450 mg./kg.) in 1,000 ml. for a 2½ per cent solution. On each occasion he received a castor oil purge the preceding evening and a tap water

enema the morning of therapy. A small amount of barium sulfate was added to the phthalofyne enema and it was followed by enough stock barium sulfate enema solution so that the first portion could be observed fluoroscopically to reach into the terminal ileum. The medicated enema was retained for a full half hour. No untoward reactions were found, but the stool specimens continued to be positive for *Trichuris* eggs and by one week after the second treatment, the estimated concentration of eggs in the stools was considered to be about the same as that noted prior to treatment.

Case No. 10 was the only one of the series in whom toxic effects on the eyes and ears were noted. He received a saline purge one evening, and was given 145 mg. of phthalofyne per kilogram by mouth the following morning while in a fasting state. Two hours later he was asymptomatic and was given a cleansing tap water enema, followed by a phthalofyne enema identical to the second enema given the Patient No. 9, that is, 450 mg./kg, in a 2½ per cent solution. Shortly thereafter he complained of "stiffness" in his hands, a sensation of feeling "cold," and of a slight headache. He ate some lunch, but shortly thereafter regurgitated a part of the food. He then rested in bed until the evening meal, of which he ate a portion and again regurgitated some of the meal. Shortly after midnight he complained of nausea, defective hearing, and pain about the eyes. Conjunctival injection, and erythema and swelling of the eyelids were observed. He was given 50 mg. of benadryl orally, and the following morning the nausea had subsided and he was able to eat and retain food. Severe photophobia and tinnitus were present at that time. Conjunctival injection and erythema of the lids were observed, though each cornea appeared normal. Visual acuity was somewhat decreased bilaterally and hearing tests, including spoken voice, whispered voice, and by audiogram, revealed moderately decreased acuity by bone and air conduction greatest in the higher frequencies.

The patient was treated with rest, a general diet, and avoidance of strong light. Three days after therapy there was less conjunctival injection, a few cells were observed in each anterior chamber, and a band of superficial punctate keratitis was seen in the right eye. By 11 days after taking the phthalofyne, the symptoms had gradually cleared and all objective findings were normal. Thereafter, no eggs of *Trichuris trichiura* were found in this man's stools during this hospital stay. A leukocyte count on the day after phthalofyne administration was 8,800; by the 11th day it was 11,300, with 5 per cent eosinophils. Routine liver function studies were normal 11 days after therapy.

This patient was recalled for examination 40 days after phthalofyne therapy and at that time he indicated that he had remained free of symptoms since his discharge from the hospital. His eyes and ears, sight and hearing, were found to be entirely normal. Three specimens of feces obtained from this patient on separate days approximately 6 weeks after therapy were examined and no eggs of *Trichuris* were found in the first 2 specimens, but a moderate number were found in the third.

DISCUSSION

The present study does not support the early enthusiasm for phthalofyne as a therapeutic agent in human *Trichuris trichiura* infestation. The drug reportedly has a margin of safety in that it tends to produce nausea and vomiting in man and dog when ingested in doses that produce severe systemic toxicity. Our patients developed some nausea and loss of gastric contents with doses which were not found to be trichuricidal and which were smaller than the most effective doses in dogs. It was only in the case of our one patient who developed eye and ear toxicity that a definite trichuricidal effect was noted. We take this to mean that enough was absorbed to produce systemic toxicity in the human host before the drug became toxic for the parasites. Even then, although the patient developed severe temporary toxicity, the parasites were not eradicated. In the case which received 175 mg./kg. by mouth without therapeutic benefit and with the production of very significant nausea and vomiting, the assumption is made that higher single oral doses would in all likelihood not be retained, and therefore would not have the opportunity to produce a significant trichuricidal effect. For the reasons stated, we feel that single dose, or 1-day therapy of this disease with phthalofyne is not feasible.

It is apparent from this study as well as others that phthalofyne definitely has trichuricidal activity, and other treatment regimens should be considered. It would seem desirable to conduct a study wherein relatively small doses are given

daily for a period of time. Before such a study is conducted on human beings, however, it would be desirable to employ such a regimen using lower primates as the test animals in order to rule out significant toxicity resulting from this mode of treatment. Further study of drugs closely related to phthalofyne may also result in the development of a therapeutic agent which is effective though relatively nontoxic.

SUMMARY

Phthalofyne (3-methyl-1-pentyn-3-yl acid phthalate), a synthetic chemical compound demonstrated to have trichuricidal activity against both *Trichuris vulpis* and *Trichuris trichiura*, was given to 10 healthy young Filipino males harboring *Trichuris trichiura*. Of 7 cases who received 100 to 125 mg./kg. by mouth, alone or in combination with the same dose given by enema, 4 had rather minimal complaints of nausea and/or vomiting and none received significant therapeutic benefit. The highest oral dose, 175 mg./kg. given to a single patient, produced considerable nausea and apparently complete regurgitation of gastric contents and no therapeutic benefit, suggesting that effective single oral doses would not be easily retained. The phthalofyne enemas, containing respectively 200 mg./kg. and 450 mg./kg. of the drug, and given one week apart to a single patient, produced no side effects and no significant therapeutic effect.

The simultaneous administration of 145 mg./kg. by mouth and 450 mg./kg. by enema resulted in moderately severe toxic effects including generalized malaise, nausea and vomiting, conjunctivitis, keratitis, and impaired auditory and visual acuity, all of which subsided within one to several days. In the one case so treated, *Trichuris* eggs were absent from the stools during a period of several days after therapy, though eggs were found in 1 of 3 stool specimens examined 6 weeks after therapy.

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ATTEMPTS TO PRODUCE IMMUNITY AGAINST THE FILARIAL WORMS OF COTTON RATS BY TRANSFER OF DEVELOPING WORMS[1]

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Previous experiments have shown that when cotton rats are given a superimposed infection of *Litomosoides carinii* 30 days after a primary infection, the worms of the superimposed infection are retarded in growth and development as compared to worms of parallel primary infections (Macdonald and Scott, 1953). For convenience we have called this retardation of growth and development an expression of "immunity." Except where specifically noted, we shall use the words immunity and immunize only in this sense. It is supposed that this expression of immunity may be based on an antigen-antibody reaction although no direct evidence is available. Work on other species of nematodes has indicated that the antigens of larval stages may differ quantitatively or qualitatively from those of adult stages (Oliver-González, 1941; Franks *et al.*, 1947). The present experiments were undertaken to determine whether worms in the fourth and fifth stages would induce immunity of the same type and degree as that produced by infective larvae. An abstract of a preliminary report of the results has been presented by Scott *et al.* (1956).

MATERIALS AND METHODS

The experiments are based on methods of transferring worms from one animal to another which were described by Olson *et al.* (1954). Donor cotton rats were infected subcutaneously according to the method described by Macdonald and Scott (1953). After periods of time varying from 15 to 28 days (a period indicated in the tables as the "age" of the worms) the rats were killed and the developing worms removed from the pleural cavity in saline solution. The worms were placed on a cover glass supported above a microscope slide on two pieces of wood. They were counted and examined to ascertain that all worms of each lot were in the same stage of development. They were flushed from the cover glass into the abdominal cavity of other cotton rats which had never previously been exposed to infection. The incisions through which the worms were introduced were closed in two layers. All incisions were subsequently found to have healed with a minimum of scar tissue and with no evidence of bacterial infection. These rats will be called "rats immunized with worms."

On the same day that the transfers were made, infective larvae were administered subcutaneously to one or more similar cotton rats in numbers approximately equal to the number of worms transferred. These rats will be called "rats immunized with larvae." From 31 to 34 days later these two series of rats were given infective larvae subcutaneously, i.e., the challenging or "test" infection. At the same time a third group of cotton rats, the "controls" were similarly infected. The experiments were done in protocols which are designated in the tables by letters. Within each protocol the rats of the three series were handled in parallel and the larvae used for final infection were dissected from mites picked at random from a single colony which had been fed once on a single infected cotton rat.

On the 24th day after the final infection the rats were killed and the worms recovered by washing first the abdominal cavity and then the pleural cavity in saline solution. On the basis of size the worms of the immunizing infection were easily separated from those of the challenging infection. The worms of the latter group were fixed, mounted and measured according to the methods described by Westbrook and Scott (1955). Each worm was examined microscopically to determine whether the final molt had begun.

The critera used for evaluating the effects of "immunity" are the mean length of the worms of each sex, expressed in tenths of millimeters, and the percentage of worms of each sex which had at least started the final molt. The statistical significance of differences between the means of the length was determined on the basis of one-tailed "t" tests (Dixon and Massey, 1951). The signifi-

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TABLE 1

Mean length of worms of the test (challenging) infection and percentage at least starting the final molt, for rats immunized with transferred worms or with infective larvae, as compared to unimmunized controls

Protocol	Number of rats	Mean number immunizing worms (W) larvae (L)	Age of worms in days	Mean adult worms recovered	Mean test larvae given	Per cent test worms recovered	Mean length of test worms in tenths of millimeters ± S.E. (number measured)		Per cent of test worms at least starting final molt	
							Male	Female	Male	Female
A	1	86 W	17	49	80	20	60* ± 10 (6)	92 ± 4 (10)	83	100
	2	Controls		—	73	50	77 ± 3 (36)	96 ± 3 (36)	100	78
	1	88 L		48	90	37	57** ± 4 (15)	70** ± 5 (17)	80*	17**
B	1	71W	26	45	81	26	70 ± 5 (9)	91* ± 7 (10)	78	70
	2	Controls		—	85	59	74 ± 2 (39)	102 ± 3 (53)	90	64
	2	72 L		17	79	41	59** ± 2 (30)	79** ± 4 (31)	81	10**
C	1	69 W	28	12	82	18	58** ± 2 (6)	91* ± 4 (9)	83	11*
	2	Controls		—	80	65	74 ± 2 (41)	104 ± 2 (58)	98	50
	2	71 L		30	81	30	67* ± 2 (19)	93* ± 5 (27)	63**	11**
D	1	51 W	27	17	70	36	64 ± 4 (6)	93 ± 6 (10)	75	7
	2	Controls		—	87	35	61 ± 2 (30)	83 ± 4 (28)	65	14
	2	49 L		25	82	48	54* ± 2 (42)	72* ± 3 (33)	31	3
E†	1	47 W	15	12	76	55	72 ± 3 (17)	95 ± 4 (24)	94	58
	2	64 L		21	83	33	41 ± 2 (25)	52 ± 3 (30)	4	7
F	1	21 W	26	5	87	36	65 ± 4 (12)	95 ± 6 (16)	38	0
	1	Controls		—	77	30	61 ± 5 (13)	81 ± 8 (10)	31	0
	2	20 L		9	76	34	50* ± 4 (20)	72** ± 4 (34)	5	0
G	1	18 W	26	11	80	51	78 ± 4 (17)	97 ± 4 (24)	100	71
	2	Controls		—	75	37	63 ± 3 (6)	78 ± 3 (30)	92	57
	1	18 L		8	75	16	43* ± 4 (5)	57* ± 10 (4)	60	0

* Difference from controls statistically significant at the five per cent level.

** Difference from controls statistically significant at the one per cent level.

† Controls died.

cance of differences between percentages was determined from one-tailed probabilities derived by Fisher's Exact Method; or where the numbers were sufficiently large, from the chi square test with Yates' correction, again using one-tailed values (Fisher, 1932).

Within each protocol all rats were of the same strain and the worms were of a strain originally derived from the same subspecies of rats. In Protocols B, C, D and F, rats and worms were of our Texas strain (Scott *et al.*, 1957) while in the other protocols the rats were from the Tumblebrook Farms strain and the worms from a strain derived from rats of the same subspecies captured near Sarasota, Florida.

RESULTS OF EXPERIMENTS

The results of the experiments are shown in Table 1 arranged by protocols in order of the number of worms transferred. Statistical analysis has shown that there were no significant differences between rats treated alike and the data for such groups are combined in the table. Mean values are shown for the length measurements and for the percentage molting. The total number of worms measured is shown in parentheses. In all cases the number of larvae given to each rat of a pair was very nearly the same, with the exception of protocol E where one rat was immunized with 52 larvae and the other with 77. A careful check has been made to ascertain that this divergent

TABLE 2

Mean length of worms in tenths of millimeters (± standard error) and percentage at least starting the final molt for various groups, with differences between groups

	1 Immunized by worms	2 Immunized by larvae	3 Unimmunized controls	1–2 Worms minus larvae	3–1 Control minus worms	3–2 Control minus larvae
Protocols A, B, C:						
Mean length, males	64 ± 4	61 ± 1	75 ± 1	3	11	14
Mean length, females	91 ± 3	82 ± 3	101 ± 2	9*	10	19
% molting, males	81	75	96	6	15*	21
% molting, females	60	12	62	48	2	50
Protocols D, E, F, G:						
Mean length, males	71 ± 2	49 ± 2	62 ± 2	22	−9	13
Mean length, females	95 ± 2	66 ± 2	81 ± 2	29	−14	15
% molting, males	80	20	69	60	−11	49
% molting, females	41	3	30	38	−11	27

* Significant at the 5-per-cent level. All other positive differences greater than 9 are significant at the 1-per-cent level.

case did not invalidate the conclusions. In each protocol the rats to which worms were transferred are listed first, the controls second and those immunized with larvae third. Thus a visual comparison can readily be made between the controls and each of the other types.

In the first three protocols listed it is obvious that the figures for rats to which worms were transferred are smaller than the corresponding figures for the control rats. In the other protocols the tendency seems to be in the opposite direction. In other words where the number of worms transferred was 69 or more, they evidently affected the growth and development of the superimposed infection. Where the number transferred was 51 or less, there was no effect. Negative values are within the range of variability usually seen in such experiments and are interpreted as having no significance since the only hypothesis adopted was that the worms might retard the growth rate. It is obvious that none of the differences noted are related to the age of the worms transferred. The worms 15 and 17 days old were in the fourth larval stage and those older were in the young adult stage. As far as these two stages are concerned, the differences in immunity seem to depend on the number transferred rather than the morphological stage of development.

In order to show more clearly the differences noted above and to provide better means for assessing their statistical validity, the data have been further combined as shown in Table 2. The measurements of all worms of each sex from each type of rat have been pooled for Protocols A, B and C, *i.e.* those in which the larger number of worms were transferred. The percentage of worms at least starting to molt has been redetermined from similarly pooled results. The results for the other four protocols, in which relatively small numbers of worms were transferred, have been similarly pooled for comparison. The differences between the values for control animals and for those immunized with infective larvae, which are shown in the last column, are all significant at the one-per-cent level. In the first three protocols where 69 to 86 worms were transferred the differences in mean length between worms from control animals and those from animals to which developing worms were transferred are also significant at the one-per-cent level. These differences were not as great, however, as the corresponding ones in the last column. The difference in the percentage of male worms which had at least started the final molt was only significant at the five-per-cent level, and the corresponding difference for female worms was not significant. In the last four protocols the differences between worms from controls and those from animals immunized with developing worms were all negative.

The principal conclusions seem to be, first, that the immunity produced by worms in the fourth larval stage or the young adult stage is not as great as that produced by an equal number of infective larvae, and second, that if enough of the fourth stage or young adult worms are transferred, some immunity does result. It seems evi-

dent that, if antigens are involved, those of the more advanced stages differ from those of the infective larvae either qualitatively or quantitatively.

DISCUSSION

An explanation of these results in terms of the mechanism involved is not possible with our present knowledge. What we are calling immunity, *i.e.*, the retarded growth of worms of an infection superimposed on an existing infection, has not been satisfactorily explained. It has been suggested that the results might be due to the formation of a precipitate in the gut similar to that demonstrated by Sarles and Taliaferro (1936) in *Nippostrongylus muris*. It has been shown that a precipitate does form at the oral opening of infective larvae of *Litomosoides carinii* when they are placed in immune serum (Scott, 1952). It is quite possible that the precipitate also forms in the gut although that has not yet been demonstrated. One hypothesis is that this precipitate is the result of an antigen-antibody reaction. The hypothesis might be extended to postulate that metabolic products of the third stage larvae act as antigens, as suggested for other nematodes by Chandler (1937), Sarles (1938), Thorson (1954) and others. On the basis of these considerations our experiments would appear to lead to the conclusion that the fourth stage larvae and young adult worms produce antigens of the same type as those produced by the third stage larvae, but in smaller quantity. There is no evidence that the older stages produce a different type of antigen, neither is there evidence against such a possibility.

SUMMARY

When fourth stage larvae or young adult worms of *Litomosoides carinii* are introduced surgically into the abdominal cavity of cotton rats, there is a retardation of growth and development of worms of a subsequent superimposed infection induced by subcutaneous introduction of infective larvae. This retardation is not so great, however, as when the primary infection is produced by subcutaneous introduction of infective larvae. Furthermore no effect is produced unless the number of fourth stage or adult worms is considerably larger than the minimum number of infective larvae required to produce this effect.

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THE STATUS OF INSECTICIDE RESISTANCE IN ARTHROPODS OF PUBLIC HEALTH IMPORTANCE IN 1956

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The major problem in the chemical control of arthropods of medical importance continues to be the development of resistance by these arthropods to the pesticides commonly employed against them. Each year the problem becomes more critical through an increase in (a) the number of resistant species, (b) the areas wherein the given species are resistant, and (c) the types of insecticides to which the species are resistant. Previous workers (Hess, 1952, 1953; Simmons, 1954; Pinotti, 1954; Brown, 1954) have discussed the general picture and significance of resistance in insects of public health importance. In this paper the present status of resistance will be considered with emphasis on the evaluation of the currently available experimental evidence for the confirmation of reported cases of resistance.

For the purpose of this discussion, resistance is defined as the ability of an arthropod population to survive exposure to dosages of a toxicant to which it formerly was highly susceptible. Two types of resistance under this category are:

1. Physiological: The ability through physiological processes to withstand a toxicant after it has entered the body.

2. Behavioristic: The ability through protective habits or behavior to avoid lethal contact with a toxicant.

Earlier summaries published on resistance have included references based on limited field observations reported in verbal or written personal communications. Where such reports have not been followed up by additional evidence or where later publications discount them, they are excluded from this resumé. In classifying the resistance reports listed herein as "confirmed" the authors based their determinations on the availability of adequate experimental data. Without such data the resistance is classified as "reported."

PHYSIOLOGICAL RESISTANCE

North America.[1] This region contains the greatest number of resistant species (Table 1), a total of 14, of which 13 are found in the United States. *Musca domestica* is the only species resistant in Canada (Roadhouse, 1953); in Mexico both the housefly (Upholt, 1950) and *Pediculus humanus humanus* (Wright and Brown, 1957) are resistant, the latter in one (Toluca) of four localities surveyed.

Beginning in 1948 the housefly throughout most of the United States became resistant to DDT. Since 1952 it has been resistant to the chlorinated hydrocarbon group in general. Organophosphorus compounds are now in widespread use against this species and with one exception no field resistant strains have developed. In a Savannah, Georgia, barn treated with malathion residual sprays in 1954 and 1955, malathion poison bait applications failed to provide control in late 1956 even though excellent abatement was achieved earlier in the season. Both physiological and behavioristic resistance were exhibited by the strain (Kilpatrick and Schoof, 1957, in manuscript; Fay *et al.*, 1957, in manuscript).

In the United States, the mosquitoes *Aedes dorsalis* (Geib, 1950), *Aedes nigromaculis* (Bohart and Murray, 1950) and *Culex tarsalis* (Gjullin and Peters, 1952) have developed resistance to DDT and other chlorinated hydrocarbon insecticides in California to the extent that malathion and parathion are now in general use by many abatement districts. In 1955 Gjullin (1956) showed that in the Delta Mosquito Abatement District where organophosphorus materials had been in use since 1951, *A. nigromaculis* larvae remained highly susceptible to malathion. However, in 1956 Gjullin and Isaak (1957) demonstrated that both larvae and adults of *C. tarsalis* were resistant to malathion but not to parathion

[1] Mexico, United States, Canada.

TABLE 1

Confirmed cases of physiological resistance to insecticides among arthropods of public health importance 1956

Species	North America	Central America and Caribbean Area	South America	Africa	Europe	Middle East	Far East	South Pacific and Hawaii
Mosquitoes:								
Aedes dorsalis	×							
Aedes aegypti		×						
Aedes nigromaculis	×							
Aedes sollicitans	×							
Aedes taeniorhynchus	×							
Anopheles gambiae				×				
Anopheles quadrimaculatus	×							
Anopheles sacharovi					×	×		
Anopheles stephensi						×	×	
Anopheles subpictus							×	
Anopheles sundaicus								×
Culex molestus					×			
Culex quinquefasciatus (fatigans)		×	×	×			×	
Culex pipiens	×							
Culex tarsalis	×							
Psorophora confinnis	×							
Psorophora discolor	×							
Flies:								
Chrysomyia putoria				×				
Musca domestica	×		×		×			×
Musca vicina				×		×	×	×
Other species:								
Blattella germanica	×							
Cimex lectularius	×				×	×		×
Cimex hemipterus							×	×
Glyptotendipes paripes	×							
Pediculus humanus humanus	×		×	×	×	×	×	

in the San Joaquin Valley where malathion had been used for only two years. Gjullin and Peters (1952) also indicated that *Culex quinquefasciatus* was resistant to several of the chlorinated hydrocarbon compounds (Table 2).

Culex pipiens was reported resistant to DDT in Ohio (Hess, 1953) and this was confirmed in the species in Massachusetts (Armstrong, 1955). In Florida, *Aedes sollicitans* and *Aedes taeniorhynchus* were found resistant to DDT and TDE (Deonier and Gilbert, 1950). Although DDT and the other chlorinated hydrocarbons are still effective in specific areas, there has been a general shift towards the use of organophosphorus compounds in salt marsh mosquito control in Florida.

In Bolivar County, Mississippi, *Anopheles quadrimaculatus* (Mathis *et al.*, 1956) and *Psorophora confinnis* (Mathis *et al.*, 1955) were found to be highly resistant to certain chlorinated hydrocarbon insecticides. *A. quadrimaculatus* adults could survive a one-hour exposure to dieldrin deposits of 200 mg. per sq. ft. with only low mortalities whereas they were easily killed by similar DDT deposits. This species also showed lowered susceptibility to chlordane and BHC residues. *A. quadrimaculatus* was suspected of becoming resistant to DDT in the lakes under treatment by the Tennessee Valley Authority (Kruse *et al.*, 1952) but later studies indicated this apparent resistance was due to causes other than resistance (Ann. Rep., T. V. A., 1955). *P. confinnis* was resistant to dieldrin in both larval and adult stages. Another rice field mosquito, *P. discolor*, also showed little susceptibility to

TABLE 2

Unconfirmed reported cases of physiological resistance to insecticides among arthropods of public health importance, 1956

Species	North America	Central America and Caribbean Area	South America	Africa	Europe	Middle East	Far East	South Pacific and Hawaii
Mosquitoes:								
Aedes aegypti			×					
Anopheles maculipennis					×			
Anopheles superpictus					×			
Culex quinquefasciatus (fatigans)	×							
Fleas:								
Ceratophyllus fasciatus			×					
Ceratophyllus londinensis			×					
Ctenocephalides felis	×		×					
Polygenis spp.			×					
Pulex irritans			×		×			
Rhapalopsyllus claviculus			×					
Xenopsylla cheopis			×					
Other species:								
Blatta orientalis					×			
Psychoda alternata	×							
Rhipicephalus sanguineus	×							
Triatoma infestans			×					

0.25 per cent dieldrin residues as an adult in 1955 (unpublished data). Since mortalities of less than 20 per cent were obtained with adults from both untreated and treated rice field areas, the low susceptibility presumably could reflect a natural resistance.

The German roach, *Blatella germanica*, initially showed resistance to chlordane in Texas (Bedingfield, 1952; Heal *et al.*, 1953) and since then resistant populations have been reported throughout the southern United States as well as in several northern cities.

Resistance of the bed bug *Cimex lectularius* to DDT is limited in distribution, the only confirmed record being from Columbus, Ohio (Anon., 1953). In Florida, the midge, *Glyptotendipes paripes*, lost its susceptibility to both BHC and EPN after one year's successful control with each material (Lieux and Mulrennan, 1956).

Other species reported resistant to chlorinated hydrocarbon insecticides are the filter fly, *Psychoda alternata*, (Bruce and Decker, 1950); the brown dog tick, *Rhipicephalus sanguineus*, (Hansens, 1956); and the cat flea, *Ctenocephalides felis*, (Kilpatrick and Fay, 1952). Despite numerous reports of the failure of DDT, chlordane, and other chlorinated hydrocarbons to control field infestations of *C. felis*, the species has never been shown to be resistant to these pesticides under laboratory conditions.

Central America and Caribbean Area. Only two species of mosquitoes have been established as being resistant to DDT in this region. In 1954 *Aedes aegypti* larvae were reported as possibly resistant in Trinidad, British West Indies (Gillette, 1954), and in 1955 laboratory tests at Trinidad (Gillette, 1955) and at Savannah, Georgia, (Fay, 1957 in manuscript) confirmed it. The later tests demonstrated that the adults also were resistant to DDT residues. Unconfirmed verbal reports suggest that resistance may be present in this species in Antigua, Dominican Republic, and Puerto Rico. In San Juan, Puerto Rico, Casta (1956, in manuscript) showed *Culex quinquefasciatus* larvae to be resistant to DDT and dieldrin. The latter gave no kill at dosages as high as 0.1 ppm while DDT yielded mortalities of 0 to 30 per cent at 0.25 ppm.

There are many unconfirmed reports of housefly resistance in this area, and it undoubtedly exists; but the authors have not seen any published references on this subject.

South America. Resistance to DDT by *C. quinquefasciatus* (*fatigans*) larvae has been confirmed (Blasquez *et al.*, 1956, unpublished) in Venezuela. Tests showed 87 per cent kill of a field strain with a dosage of 10 ppm of DDT as compared to 100 per cent mortality of a laboratory colony at 0.5 ppm. This species also has been reported resistant in Brazil, Colombia and British Guiana (Pinotti, 1954) and in Ecuador (Levi-Castillo, 1953). In British Guiana, de Caires (1955) conducted laboratory tests which showed *C. quinquefasciatus* (*fatigans*) to have a low level of susceptibility to DDT which he considered to be natural rather than acquired. From the available evidence this opinion presumably applies to the species in the other countries. Field-collected *Aedes aegypti* larvae in Venezuela were shown by Blasquez (1956, unpublished) to be resistant to DDT in laboratory tests in which dosages of 0.1 and 5 ppm produced mortalities of 7.6 and 92.8 per cent respectively. Unfortunately no laboratory strain was available for comparison with this field strain.

Wright and Brown (1957) list Peru and Chile as sources of resistant *P. humanus humanus* but the species was not resistant in all the areas tested. *M. domestica* was reported by Pinotti (1954) to be resistant throughout South America and confirmatory evidence was presented by Schiavi (1949) and Lopez Fernandez (1950).

As Table 2 indicates, seven species of fleas (Saenz-Vera, 1953) were reported to be resistant to DDT in Ecuador based on failure of this pesticide to control field infestations.

In Chile, *Triatoma infestans* was indicated by Neghme and Silva (1948) to be resistant to DDT. This resistance possibly may be "natural" since DDT has not been employed successfully for the control of this species. Hack and Romaña (1953) also reported DDT to be ineffective against this species in laboratory tests in Argentina.

Africa. Musca vicina in Egypt was shown to be resistant to chlorinated hydrocarbon insecticides by Gahan and Weir (1950) but information is lacking on the susceptibility of this species in other parts of Africa.

In the Belgian Congo, *Chrysomyia putoria*, a species commonly found breeding in excrement in privies, has been established as resistant to chlorinated hydrocarbon and organophosphorus insecticides by Bervoets *et al.* (1957, unpublished). After initial success with BHC treatments against larval infestations this toxicant gave unsatisfactory control. Replacement of BHC with Diazinon again re-established good abatement but during the second year of application the species also exhibited resistance to this compound. Tests showed that with larvae from the treated area a dosage of 363 ppm of Diazinon was required to obtain a kill equivalent to that produced by 65 ppm against larvae from an untreated zone. Dosages of Chlorthion (250 ppm), malathion (200 ppm) or parathion (250 ppm) gave 100 per cent kill of susceptible larvae in 5 hours whereas against the Diazinon resistant strain 42.5, 1.9 and 9.0 per cent, respectively, survived 16 hours exposure, thus indicating cross-resistance within the compounds tested. This record is the first instance of resistance to chlorinated hydrocarbon and organophosphorus insecticides in the blow fly group.

C. quinquefasciatus (*fatigans*) and *Anopheles gambiae* have been reported resistant to chlorinated hydrocarbon insecticides. On the island of Reunion, Hamon (1953) established the resistance of *C. quinquefasciatus* (*fatigans*) larvae to DDT and BHC. In Northern Nigeria, *A. gambiae* was shown to develop a high resistance to dieldrin deposits within 18 months after its initial exposure to this pesticide (Elliott and Ramakrishna, 1956). The dieldrin resistant strain remained susceptible to DDT. Studies by Davidson (1956) demonstrated that this resistant strain was composed of susceptible, hybrid and resistant segregates, the resistance being inherited on a simple Mendelian basis.

DDT-resistant body lice were recorded from Egypt (Busvine, 1953; Hurlbut *et al.*, 1954), and Wright and Brown (1957) list DDT-resistant strains from Angola, Cameroons, Ethiopia, French Guinea, Senegal, Madagascar, Nigeria, Sudan and the Union of South Africa.

Europe. Missiroli (1947) and Saccà (1947) first reported DDT-resistance in *M. domestica*. Since then this species has come to be considered resistant in all areas where chlorinated hydrocarbon insecticides have been employed. After the failure in Denmark of DDT and related compounds for fly control (Wichmand, 1953), parathion, Bayer Compound 21/199 (Resitox) and Diazinon were utilized successfully for this purpose. However, in the summer of 1955 loss of control was reported from several areas. Laboratory tests showed definite resistance to Diazinon and Bayer 21/199 in several field collected strains

(Keiding, 1956). These data were the first to demonstrate that *M. domestica* could develop resistance to an organophosphorus compound under field conditions.

Culex molestus was the first mosquito reported resistant to DDT (Mosna, 1947) while *Anopheles sacharovi* in Greece gave the initial confirmation that a malaria vector could lose its susceptibility to this toxicant (Livadas and Georgopoulos, 1953). Larvae of the latter species were reported resistant to dieldrin and chlordane by Belios and Fameliaris (1956). Other reports of possible anopheline DDT-resistance also concern Greece—*Anopheles maculipennis* and *Anopheles superpictus* (Livadas, 1955).

In surveys of six Eurpoean countries (Wright and Brown, 1957), evidence was found of resistance in *P. humanus humanus* in only one of the 27 localities. Livadas and Petrides (1955) concluded from a survey in Greece that louse resistance was absent; however their data indicate slight resistance in two of the 31 samples tested (Wright and Brown, 1957). Nicoli and Sautet (1955) also reported resistant lice on transients at Marseilles, France, one of which was a native from the Var area.

Mosna (1954) showed *C. lectularius* to be resistant to several chlorinated hydrocarbon insecticides in Italy. Resistance was reported in *Pulex irritans* in Greece (Hadjinicolaou, 1952) and in *Blatta orientalis* in Italy (Ricci, 1948).

Middle East[2]. In Israel, *M. vicina* has developed resistance to DDT (Davidovici *et al.*, 1950; Silverman and Mer, 1952). Unconfirmed reports indicate that such resistance is general over the region.

Resistance to DDT in *A. sacharovi* in Lebanon was first noted by Garrett-Jones and Grammiccia (1954). Busvine (1956) confirmed this resistance in 1955. In Saudi Arabia, Daggy and Peffly observed the gradual appearance of *A. stephensi* in DDT-sprayed houses in 1954–55 and suspected the development of resistance which was confirmed by Davidson (1955).

Bed bugs and human body lice are resistant to DDT in the Middle East. Wright and Brown (1957) listed resistant strains of *P. humanus humanus* in Iran, Syria, Turkey and Jordan. Levinson (1953) recorded a DDT-resistant strain of *C. lectularius* in Israel while Cwilich *et al.* (1957) found a BHC-resistant strain in the same country.

Far East. DDT-resistant *M. vicina* have been detected in Japan (Byers *et al.*, 1956) and a BHC-resistant strain is recorded from Ceylon (Macdonald, 1954). The species also is reported to be resistant to BHC in Hong Kong (Romer, 1953). Aside from these references, there are no reports of extensive resistance in this species in the Far East.

Pal *et al.* (1952) found resistance in *C. quinquefasciatus* (*fatigans*) in India (Delhi State). Only 9 per cent of the females from a sprayed town died when exposed for 30 minutes to 50 mg. of DDT per sq. ft. as compared to 90 per cent kill of females from an untreated area. Rajagopalan *et al.* (1954) observed BHC resistance in this species at Kumbakonam in South India. In Malaya, Reid (1955) reported BHC to give unsatisfactory control of larvae of *C. quinquefasciatus* (*fatigans*) after 2 years of successful abatement. Laboratory tests confirmed the resistance to BHC; cross resistance to dieldrin was evident, but none to DDT.

Despite widespread residual spraying with DDT, anopheline resistance to this pesticide has been noted in only two localities. In 1955, Rajagopalan *et al.* (1956) found larvae of *A. stephensi* from wells treated in Erode Urban, India, with DDT since 1947 to be resistant to that toxicant. Sharma and Krishnamurthy (1957) reported adult *Anopholes subpictus* from Delhi State, India, to be resistant to DDT in a village treated with this pesticide for 10 years.

Laboratory tests in Hong Kong, China, showed *Cimex hemipterus* to have a low degree of resistance to DDT but none to pyrethrins or BHC (Medical and Health Services, 1955, unpublished). *C. hemipterus* also was reported by Rao and Halgeri (1956) to be resistant to DDT in Bombay State, India. Residual sprays of DDT for malaria control were observed to be ineffective against bed bugs in 5 localities, the earliest record being in 1951. Laboratory tests in 1952 and 1955 with bed bugs collected in Poona City revealed that 10 minute exposure to 1, 2, and 5 per cent DDT dusts (talc) gave zero per cent mortality with specimens in 1955 as compared to 41, 64, and 76 per cent kills with specimens in 1952. Thirty-minute exposure of the strain in 1955 to a 50 per cent dust yielded a maximum kill of 54 per cent only.

DDT-resistant lice have been found in Japan,

[2] Includes Turkey, Syria, Lebanon, Israel, Saudi Arabia, Iran and Iraq.

China and Korea. The data for Korea represented the first confirmed report of resistant lice (Hurlbut *et al.*, 1952). The species also is resistant to DDT in Japan (Kitaoka, 1952; Barnett and Knoblocks, 1952; Wright and Brown, 1957). The latter listed DDT-resistant strains in all nine towns surveyed, and in several localities the lice were resistant to BHC and pyrethrum. A DDT-resistant strain is also listed from Hong Kong (Wright and Brown, 1957).

South Pacific[3] *and Hawaii.* Byers *et al.* (1956) showed that *M. vicina* from Okinawa were resistant to DDT but susceptible to lindane, dieldrin and malathion. In New Zealand, Harrison (1951) found DDT-resistance in *M. domestica* and Kerr and Venables (1955 unpublished) detected DDT resistance in field-collected strains in Australia.

Only one mosquito, *Anopheles sundaicus*, is recorded as resistant in this region. Crandell (1954) found this species was not controlled by DDT residual treatments in the Djakarta coastal area and in Tjirebon. Chow and Soeparmo (1956) confirmed that the species also was DDT-resistant in Semarang. All three localities have a history of the use of larvicidal treatments for the control of *A. sundaicus*.

The initial occurrence of DDT resistance in *C. lectularius* was established by Johnson and Hill (1948) in Hawaii. In 1953, Chen *et al.* (1956) in Formosa demonstrated the first instance of DDT resistance in *C. hemipterus*. Specimens from an unsprayed village exposed to 5 per cent DDT impregnated cloth showed 100 per cent mortality after 2 days exposure; none of the specimens from the treated area were dead after 4 days of exposure to similarly treated cloth.

BEHAVIORISTIC RESISTANCE

Relatively few confirmed cases of behavioristic resistance have occurred since the general application of chlorinated hydrocarbon insecticides began. Part of the difficulty in assessing this type of resistane in the field is the scarcity of information on arthropod behavior before chemical control was initiated.

Muirhead-Thomson (1947) first reported what appeared to be behavioristic resistance to DDT by *A. gambiae* and *Anopheles melas*. Later studies by Hadaway (1950) indicated that this apparent behavioristic resistance by *A. gambiae* may be a natural response. The only authenticated instance of this type of resistance in mosquitoes was reported by Trapido (1952) on *Anopheles albimanus* in Panama. This worker found that after 6 to 8 years of DDT treatments, the densities of *A. albimanus* in houses began to increase. However, laborabory tests (Trapido, 1954) confirmed the continued susceptibility of the species to DDT residues. Despite the increased densities of the mosquitoes in treated houses, malaria rates remained low, presumably because the treatments still were effective in reducing the number of specimens feeding upon man, apparently the result of a development of a hypersensitivity of the species to DDT.

In South America, Simmons (1954) listed *A. albimanus*, *Anopheles pseudopunctipennis*, and *Anopheles albitarsis* as behavioristically resistant to DDT in Venezuela but Pinotti (1954) reported no anopheline resistance in that country. *Anopheles darlingi* was indicated by Simmons (1954) as being behavioristically resistant in British Guiana, Venezuela, Argentina and Brazil but with the exception of Brazil, Pinotti (1954) discounted these reports. In Brazil, Bustamante *et al.* (1951 and 1952) recorded observations which indicate the probable existence of behavioristic resistance in *A. darlingi*.

In houseflies, several reports suggest that after the use of DDT, a change occurred in the resting habits of the species which increased its chance of survival. Studies by Kilpatrick and Quarterman (1952) and Maier *et al.* (1952) with *M. domestica* in rural and urban areas showed that this species tended to rest out-of-doors at night during the warmer periods of the year. Similar observations on *M. vicina* were made in Israel by Silverman and Mer (1952). With both species, no similar sutdies had been made prior to the development of DDT-resistant strains; however, the opinion of the authors and associates is that houseflies rested indoors before the use of residual sprays.

During 1956, behavioristic resistance of *M. domestica* to malathion baits was demonstrated by Kilpatrick and Schoof (1957, in manuscript). Housefly populations which readily fed upon malathion baits for 12 weeks suddenly showed an aversion to contacting the bait. The use of other baits containing malathion likewise were ineffective. Field and laboratory tests confirmed the lack of attractiveness of malathion baits to this strain (Fay *et al.*, 1957, in manuscript).

[3] Includes Okinawa, Formosa, East Indies, Philippine Islands and Australasia.

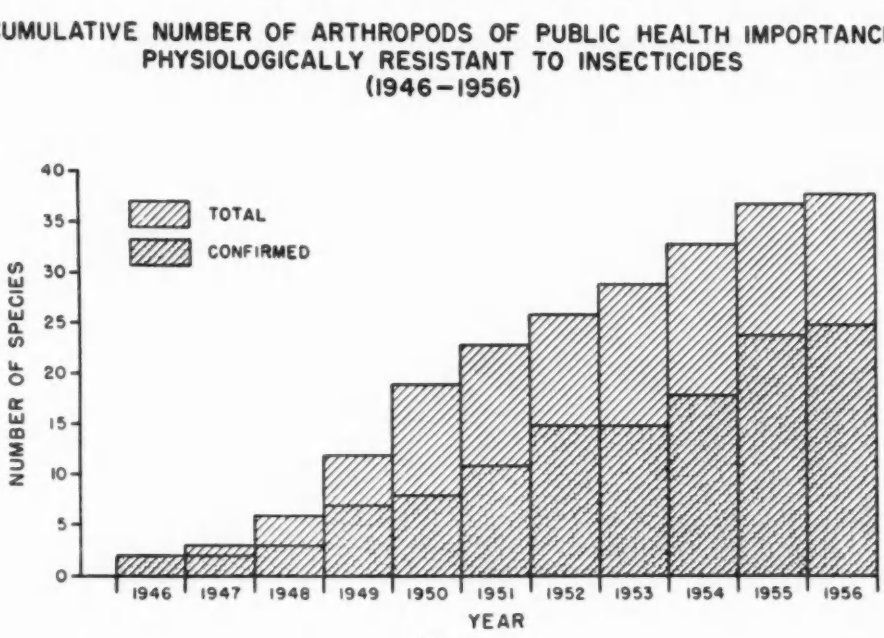

FIG. 1

DISCUSSION

The annual cumulative increase in the number of physiologically resistant arthropod species, confirmed and reported, is shown in Figure 1. The distribution of these species is given in Tables 1 and 2. These listings do not include several species mentioned previously by other authors. Such species, as well as those listed in Table 2, undoubtedly represent some bona fide instances of resistance. However, the absence of adequate supporting data in published form makes it difficult to judge the authenticity of the reports. Workers in areas where resistance is reported but is unconfirmed, are urged to investigate these claims and establish their validity by experimental proof. The authors feel that the problem is too important to permit unsubstantiated reports to gain an unwarranted measure of credence by remaining in the literature indefinitely and being repeatedly quoted by subsequent reviewers.

Likewise, in all new instances of suspected resistance, supporting experimental data should be obtained before statements on the occurrence of such resistance are published. Brown *et al.* (1954) made a noteworthy contribution in this respect by disproving the existence of DDT-resistant mosquitoes in Southern Ontario. More studies of this type are needed. The World Health Organization and the United States International Cooperation Administration currently are in the process of providing resistance kits to field projects to assist in the detection of resistance. The potential value of such assistance is reflected in the results of the body louse survey (Wright and Brown, 1957).

As indicated in Table 1, resistance has been found in numerous species of arthropods of public health importance throughout all parts of the world. However, only houseflies and the body louse appear to be resistant on a global basis. Nonetheless, in specific areas, these species can still be controlled by chlorinated hydrocarbon insecticides. With other species the geographic extent of resistance is much more limited, e.g., in the United States, *C. tarsalis* is resistant to DDT and related compounds in California but still is susceptible to these pesticides in other states. In general, resistance has appeared in areas where insecticides have been used most extensively. However, it cannot be predicted if and when resistance to a given toxicant will

appear in any species. This uncertainty, together with the undetermined benefit of using alternate pesticides in an area, indicates that residual applications of the same insecticide should be continued in a zone as long as such remain effective.

Based on studies showing that exposure of both larval and adult stages of *M. domestica* to the same pesticide hastens the onset of resistance to chlorinated hydrocarbon insecticides (Bruce and Decker, 1950), the suggested control procedure has been to use the organophosphorus toxicants as larvicides and reserve the chlorinated hydrocarbon insecticides for adulticiding purposes. However, recent studies (March *et al.*, 1956) show that selection of susceptible strains of houseflies by exposure to Diazinon or parathion induced high levels of resistance to DDT and dieldrin in these strains. Such data suggest that the field use of organophosphorus compounds as mosquito larvicides could possibly produce resistance to the chlorinated hydrocarbon insecticides in the adult populations. Both laboratory research and field observations are urgently needed to elucidate this problem.

The widespread development of resistance to chlorinated hydrocarbon insecticides has caused a general shift to the use of organophosphorus pesticides in many areas. With the exception of resistance of *M. domestica* in Denmark, of *C. putoria* in Leopoldville, Belgian Congo, and of localized resistance of *M. domestica*, *C. tarsalis* and *G. paripes* in the United States, there has been little indication that organophosphorus compounds are losing their general effectiveness against arthropods of medical significance. However, these initial reports of resistance to the organophosphorus compounds may be only a forerunner of future extensive difficulties.

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APPLICATION OF RADIOISOTOPES TO THE STUDY OF THE METABOLISM OF FRESH-WATER SNAILS (GASTROPODA-PULMONATA)

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The important results obtained by the use of radioactive isotopes in the solution of many medical and biological problems made it seem worth while studying the metabolism of different ions in fresh-water snails. These molluscs were selected because of their role in tropical medicine as intermediate hosts of *Schistosoma* which is the cause of much disease in many overseas provinces of Portugal, especially Mozambique (Portuguese East Africa). This paper comprises the results of several investigations in which a few of the isotopes more frequently used in medicine and biology (all with a short half-life between 12 hours and 14 days) were selected for their suitability to the planned experiments, and because they were easy to acquire and to manipulate.

MATERIALS AND METHODS

The isotopes used were P^{32}, I^{131} and Cu^{64}; Ca^{45} (with a longer half-life of 152 days) is at present also under study. In the first series, in which P^{32} was used as a label, the radioactivity of the bath in which the snails were placed was measured daily, since it was not known whether they would absorb the phosphorus from the solution. When this was ascertained, the procedure could be simplified and the autoradiographic technique applied.

1. Metabolism of phosphorus, using P^{32}

A number of adult snails, *Helisoma duryi normale*, were prepared and 8 snails were placed in each of 3 cylindrical glass tubes 4 cm. in diameter and 8 cm. high, with a capacity of 100 ml. Into each tube was poured 60 ml. of tap water (which contained Ca and Cl) and 0.4 ml. of sodium orthophosphate solution made up in such a way that this amount contained 40 μg of radioactive phosphorus (P^{32}). The usual precautions in handling an emitter of pure beta particles were taken to avoid direct contact of the operator with the radioactive solution.

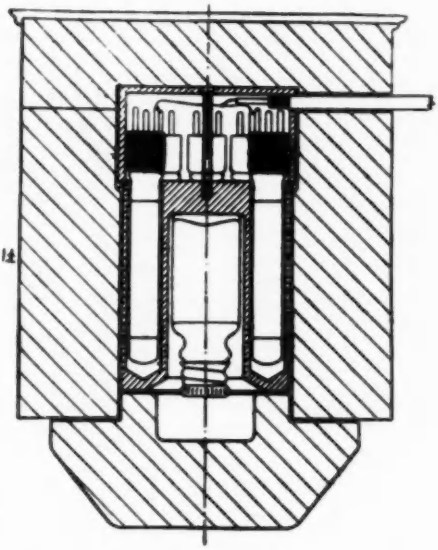

FIG. 1. Diagram of the system of Veall and Baptista. In the center stands the flask containing the substance to be measured, surrounded by the gamma detectors of which only 2 may be seen.

The specific activity of the whole solution was measured by the Veall and Baptista method (1954). This method consists fundamentally of an assembly of 8 Geiger-Müller detectors for gamma radiation, placed in a ring surrounding the cylindrical cavity where the sample to be measured stands. A diagrammatic section of such an assembly is shown in Fig. 1. While P^{32} is a pure beta emitter, the great energy of its particles ($\bar{E}$ = 1,701 *MeV*) makes it possible to measure its radioactivity with gamma detectors through what is called the "Bremsstrahlung."[1] In this as-

[1] Bremsstrahlung is the name given to secondary X radiation produced when an electron is deflected in the field of an atomic nucleus; under these conditions the β particles (electrons) when crossing

FIG. 2. Observer introducing a sample into the system to be measured. In the rear, may be seen a scaler and a source of stabilized tension.

sembly (Fig. 2) the activity of one microcurie (μc) of P^{32} in an aqueous solution with a volume of one ml. produces 120 pulses per minute, from which it could be computed that the solutions in the tubes containing the snails had a specific activity of 7.42 μc/ml.

The absorption of phosphorus by the snails was calculated quantitatively by observing the decrease in radioactivity of the liquid with time, after correcting for the natural decay of P^{32} which has a half-life of 14.3 days.[2] This was done by taking daily samples of 10 ml. of the solution from each tube, measuring the radioactivity and restoring the samples to their respective tubes. In such a system the baseline, or measure of radioactivity at the time the snails are introduced, as well as subsequent measurements, must take into account also the "dead time"[3] and the background radiation due to cosmic rays, air contamination, etc.

RESULTS

The results obtained in one of the tubes are illustrated in Fig. 3 (the other tubes being identical) and shows a 22 per cent decline in radioactivity in 5 days. If we ignore the phosphorus that might be returned to the solution in the feces, this decrease in observed activity is the measure of the percentage of the sodium orthophosphate absorbed by the snails. This figure cannot be accepted unconditionally since, besides the phosphorus possibly returned in the feces, we know that the calcareous substance of the snail's shell may absorb ions just as calcite crystals do, as observed by one of us (Baptista). Investigation showed that the radioactivity of the soft parts of the snail was about four times that of the shell. As for the feces, the liquid in the tube was centrifuged after 19 days and the residue washed in distilled water and the phosphorus

the substance originate the Bremsstrahlung and may then be measured.

[2] A_o being the initial activity of the radioisotope, the activity, (A), after the elapsed time t is given by the formula:

$$A = A_o \cdot e^{-\frac{0.693t}{T1/2}},$$

in which e is the base of the Napierian logarithms ($e = 2.718$) and $T^1{}_2$ is the half-life of the considered isotope (in this case $T^1{}_2 = 14.3$ days).

[3] *The dead time* or *paralysis* may be defined approximately as the minimum time elapsed between 2 detected counts, during which interval no particle is registered by the counting system. The counts registered per time unit being N_c, the true number of counts (N), in the same time unit is given by $N = \frac{Nc}{1 - N_c\gamma}$, in which γ is the dead time of the counting system.

content determined. This proved to be relatively small (maximum 2,200 p.p.m.) as compared with that of the solution (maximum 8,550 p.p.m.), with correspondingly low radioactivity.

Autoradiographic study. Since it was shown that the snails metabolized the phosphorus, sections were made of their bodies and by autoradiography a study was made of the distribution and localization of phosphorus in the exposed specimens. For this, the "stripping technique" of Pelc (Doniach and Pelc, 1950) was used. The snails were anesthetized with ether and killed in hot water; they were then fixed in 90 per cent alcohol or 8 per cent formaldehyde and imbedded in paraffin. Sections of various thickness were made and mounted on slides treated with gelatin. By use of a "window detector" (4 mg./cm^2) it was found possible to obtain good images of the radioactive material with an exposure of about 4 days. For this Kodak autoradiograph plates were used with an emulsion thickness of 4 μ. After development and fixation, the slides, stained with hematoxylin-eosin, were superimposed on the dried plates and mounted in Canada balsam under a cover-glass.

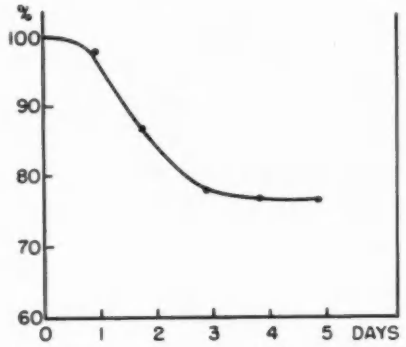

FIG. 3. Diagram showing the decrease of phosphorus from 100 to 78 per cent in 5 days.

The autoradiographs revealed that the ion-phosphate had been absorbed by the intestine, and was diffused in various organs with a concentrated deposit in the hepato-pancreas. A characteristic photomicrograph made from one of these autoradiographs is shown in Fig. 4.

II. Metabolism of iodine, using I^{131}

The method of studying the metabolism of iodine was the same as that described for phosphorus. Solutions of potassium iodide were prepared containing the radioactive isotope I^{131},

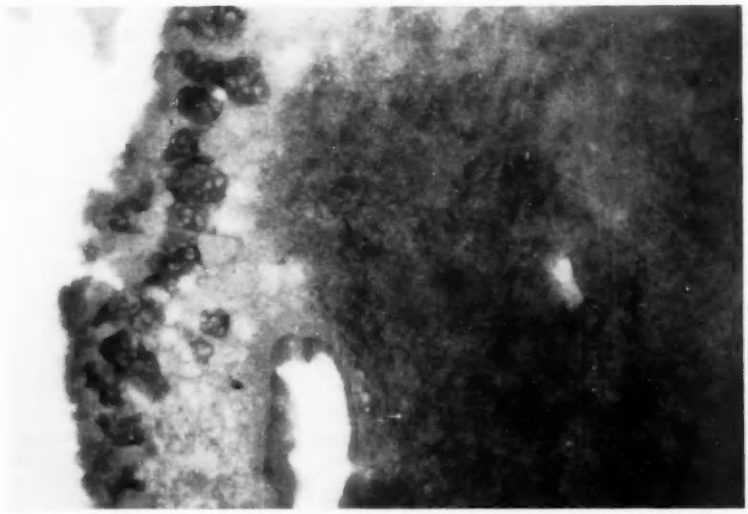

FIG. 4. Photograph of part of the hepatopancreas (*Helisoma durgi normale*). In the area of the lobules, phosphorus may be seen in great quantities in the parenchymatous cells. The clearest parts correspond to inter lobular spaces of connective tissue almost without phosphorus. The internal part of the organ shows greater accumulation, perhaps as reserve and deposit.

FIG. 5. A fragment of the prostate (*Helisoma duryi normale*). The black dots representing I^{131} may be seen on the left side; the lack of dots on the other side is due to the absence of radiographic film (R).

which emits both beta and gamma radiations, and has a half-life of 8.0 days. Eight adult snails of the same species (*Helisoma duryi normale*) were put into the cylindrical tubes already described, with 60 ml. of H_2O to which enough isotope was added to produce a specific activity of 6.67 μc/ml., giving a total activity of 400 μc.

Autoradiographs from snail sections revealed the iodine diffused through various organs, with some concentration, though not as much as that observed with P^{32}, in the hepato-pancreas. A characteristic photomicrograph is shown in Fig. 5.

III. Metabolism of copper, using Cu^{64}; mechanism of the molluscicide action of copper sulfate

In this test snails of four species were used: *Helisoma duryi normale*, *Planorbarius corneus*, *Biomphalaria pfeifferi* and *Australorbis glabratus olivaceus*. This study was particularly interesting in view of the molluscicidal role of copper sulfate, the histopathologic action of which had been previously studied by two of the writers (Fraga de Azevedo and Carvão Gomes, 1956a).

Copper sulfate is an effective molluscicide in dilutions of one p.p.m. (Mozley, 1952). Because of its short half-life of 12.8 hours, it was necessary to use a higher concentration of the radioactive isotope Cu^{64} than of the other isotopes in order

TABLE 1

Activities lower than 2 mC/ml

Tube No.	Activity	Volume of initial solution diluted up to 50 ml of water	Weight of copper sulphate	Concentration
1	1 mC/ml	2.5 ml	0.2510 g	0.0050 g/ml
2	0.5 mC/ml	1.25 ml	0.1255 g	0.0025 g/ml
3	0.250 mC/ml	0.625 ml	0.0628 g	0.0013 g/ml

TABLE 2

Activities higher than 2 mC/ml

Tube No.	Activity	Volume of initial solution diluted up to 50 ml of water	Weight of copper sulphate	Concentration
4	4 mC/ml	10 ml	1.004 g	0.0201 g/ml
5	2 mC/ml	5 ml	0.5002 g	0.0101 g/ml

TABLE 3

Relation activity/weight of snails

Tube No.	Number of snails	Weight of snails without shells, in centigrams	Activity of all snails (p.p.m.)	Activity per centigram (p.p.m./cg)
6	6	56	902	16.1
7	6	35	582	16.6
8	6	72	1142	15.9

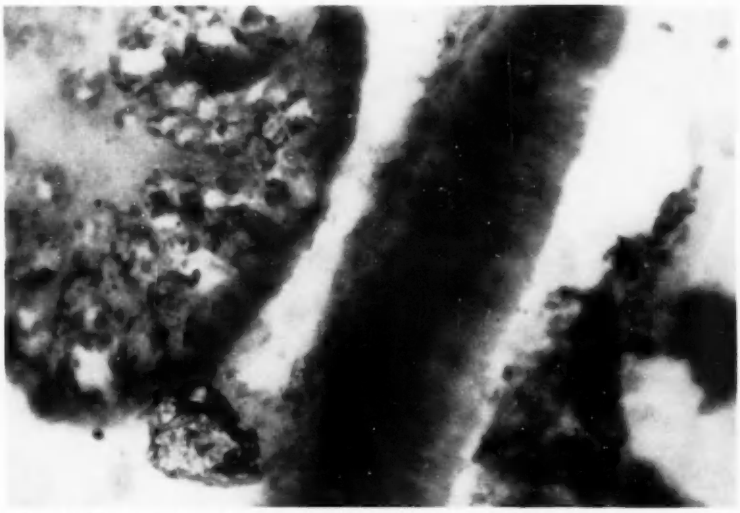

FIG. 6. Part of the prostate and a fragment of the intestine of *Helisoma duryi normale* in which the black dots correspond to the imprint by Cu^{64} on the superposed film.

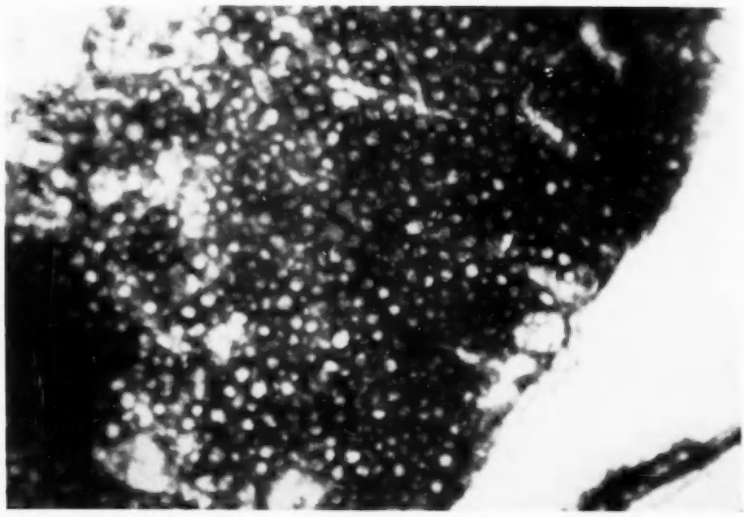

FIG. 7. Section of the hepatopancreas of *Planorbarius corneus* full of Cu^{64}, which gives it an aspect almost completely black.

to obtain sufficient localization of Cu in the various organs to be detected by autoradiography. However, the deaths of snails in this experiment were due exclusively to the toxic action of the $CuSo_4$ and not to the action of radiation, as shown by a preliminary study reported elsewhere (Fraga de Azevedo and Carvão Gomes, 1956b) on the resistance of these snails to ionizing radiation.

Various radioactive solutions were prepared starting with 2 grams of Cu^{64} in metallic form with a total radioactivity of 1.0 curie. Disintegration occurs by: a) emission of beta particles (0.57 *MeV*

of maximum energy of 19%) leading to the stable isotope Zn^{64}; b) by positronic emission (0.66 *MeV*, 39%); and by electronic capture (42%) leading to the stable isotope Ni^{64}. (Since both beta and gamma rays are produced, special care must be taken in handling activities as great as one curie.) The metallic copper was transformed into copper sulfate by the action of chemically pure sulfuric acid, producing 5.0204 gm. of labeled $CuSo_4$. Since the initial solution had a volume of 50 ml., the concentration of $CuSo_4$ was therefore 0.1004 gm./ml., and the specific activity was 20 millicuries/ml. Successive dilutions were prepared as shown in Tables 1 and 2.

The method of exposing the snails was exactly the same as in the previous experiments, but the 8 snails comprised 2 each of the 4 species mentioned above. All the snails died within 18 hours from the toxic action of the $CuSo_4$.

It was then decided to test the possible interference (adjuvant or inhibitory) of other chemical compounds with copper sulfate, reproducing the conditions which sometimes occur in nature in the habitats of these snails. Three tubes were set up (numbered 6, 7 and 8), each with 6 snails of the species *Helisoma duryi normale*, chosen because it is easy to obtain in Portugal. Into each tube was put a solution of radioactive copper sulfate prepared by diluting 10 ml. of the initial solution containing 0.1004 gm./ml. of $CuSo_4$ with 30 ml. of distilled water. The resulting 40 ml. were equally divided among the 4 tubes, so that each contained $\frac{0.1004 \times 10}{4} = 0.205$ grams of $CuSo_4$. One of these tubes was kept as a control; to the second was added about 300 mg. of sodium orthophosphate and to the third the same amount of sodium sulfate. The fourth tube was discarded because of an accident. The weight of the added chemicals was about that of the copper sulfate in each tube. The radioactivity of the snails was measured as in the other tests with the results shown in Table 3. The addition of these compounds did not apper to produce a significant difference.

Sections were made of the snails exposed in tubes 1–5 (Tables 1 and 2) and photomicrographs of the autoradiographs show a diffusion of copper into almost all the organs, with a toxic action on protoplasm such as that observed by histologic study (Fig. 6 and 7).

SUMMARY AND CONCLUSIONS

Fresh-water snails (*Helisoma duryi normale*) were placed in solutions containing phosphorus, iodine or copper tagged with the respective isotopes P^{32}, I^{131}, and Cu^{64}. It was shown by means of autoradiographs of the snail tissues that the radioactive compounds were absorbed by the intestinal tract and became diffused through the various organs, with a tendency to concentrate in the hepato-pancreas, especially in the case of phosphorus. The toxic action of copper on the protoplasm was observed, confirming a previous histopathological study. Addition of a phosphate or a sulfate, which are often found in natural habitats of snails, did not interfere with the molluscicidal action of copper sulfate.

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THE PATHOLOGY AND PATHOGENESIS OF FATAL INFECTIONS DUE TO AN ACANTHOCEPHALID PARASITE OF MARMOSET MONKEYS

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Ten squirrel-marmoset monkeys, *Saimiri o. orstedii*, were purchased in May, 1953, from an animal dealer in David,ep Rublic of Panama, for use in experiments in the transmission of pathogenic fungi. The animals were captured in the forests of Chiriqui Province and held by the dealer for about ten days before being shipped to the Gorgas Hospital Laboratory by air freight. They were kept in isolation for five days after arriving at the laboratory and appeared to be active, healthy specimens. During this period blood cultures were done on each monkey and were negative for organisms. Eight of the marmosets were used in experiments while two were maintained as controls. The squirrel-marmoset had not been used previously at the laboratory.

Eight experimental monkeys were allowed to breathe for various periods of time in a special chamber into which an atomized spray of saline solution was introduced containing the pathogenic yeast, *Cryptococcus neoformans*. The control animals were exposed to saline droplets which did not contain fungi. Afterwards the animals were maintained in separate cages, which they had occupied since their arrival at the laboratory. They were fed on native fruits, bread, bits of cooked meat, and given supplemental multivitamin drops two or three times a week. Four of the marmosets, including both of the controls, died as a result of infection by an acanthocephalid parasite, *Prosthenorchis elegans*. The remaining animals were not infected by this parasite; at autopsy each showed a diffuse brancho-pneumonia. Histopathological findings were nearly identical for all helminth-infected marmosets; the observations on this group are summarized as follows.

OBSERVATIONS

Marmoset A-14 died suddenly just sixteen days after arrival at the laboratory. The control animals (A-13, A-19) died on the 29th and 27th days respectively. The last worm-infected monkey, A-18, survived thirty-three days. Table 1 summarizes the data concerning all monkeys obtained from the Chiriqui Province.

The monkeys infected by *Prosthenorchis* usually died after a period of illness lasting less than a day. They abruptly became listless, refused food, and within a few hours lay prostrated on the floor of their cages, scarcely moving. The respirations became deep and sighing, then rapid and shallow, followed by an interval of apnea. This pattern of Cheyne-Stokes respiration continued until death intervened.

The gross pathological findings at autopsy were unremarkable except for the abdominal cavity. Three monkeys had an extensive peritonitis involving the cecum, proximal colon, and patchy areas of mesentery. Only the proximal colon was thus involved in marmoset A-18. The serosal surface of the colon of these animals was studded with nodules ranging from two to six mm. in diameter. These were light yellow, smooth and shiny, with a pearly luster. These nodules were not found in the six monkeys which did not harbor *Prosthenorchis*. Perforation of one of these nodules was demonstrated at autopsy in three monkeys. On opening the large intestines, they were found to be filled with large thick worms ranging from two to four cm. in length. The proboscis of each parasite was firmly embedded in a tunnel in the wall of the bowel. Considerable pressure had to be exerted on the worms to dislodge them. At the openings of the tunnels the mucosa of the intestine was raised; it was usually normal in color, but in some areas was injected and red. The nodules of the serosa were located opposite the places where the worm tunnels penetrated the mucosa of the bowel. This same reaction has been noticed by one of us in the invasion of the serosa by *Pomphorinchus bulbicoli* in the common sucker, *Catostomus commersoni*.

Examination of histological sections of the colon revealed that the worms had destroyed the mucosa, muscularis mucosa and submucosa, and had penetrated into the smooth muscle coat of the wall in forming their tunnels (Fig. 1 and 2).

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TABLE 1

Data relating to the marmoset monkeys, Saimiri o. orstedii *observed during this study*

Animal number	Sex	Weight in grams	Total survival in lab. (days)	No. of Acanthocephala†	Use	Pathology due to *Prosthenorchis*
A-10	Male‡	305	35	None	Exp.	None
A-11	Male‡	356	35	None	Exp.	None
A-12	Male‡	310	38	None	Exp.	None
A-13	Male*	280	29	89	Contr.	Acute peritonitis with perforation.
A-14	Female	400	16	110	Exp.	Acute peritonitis with perforation.
A-15	Male‡	315	35	None	Exp.	None
A-16	Female‡	347	17	None	Exp.	None
A-17	Male*‡	275	35	None	Exp.	None
A-18	Female	415	33	93	Exp.	Localized acute peritonitis.
A-19	Male*	200	27	45	Contr.	Acute peritonitis with perforation

* Subadult monkeys.

† Numbers of Acanthocephala counted directly and by piecing out the fragments of worms cut in taking sections.

‡ Animals completely autopsied; cultures for fungi and bacterial pathogens made from vital organs and blood; numerous histological sections examined for *Cryptococcus neoformans* which was recovered but none died of cryptococcosis.

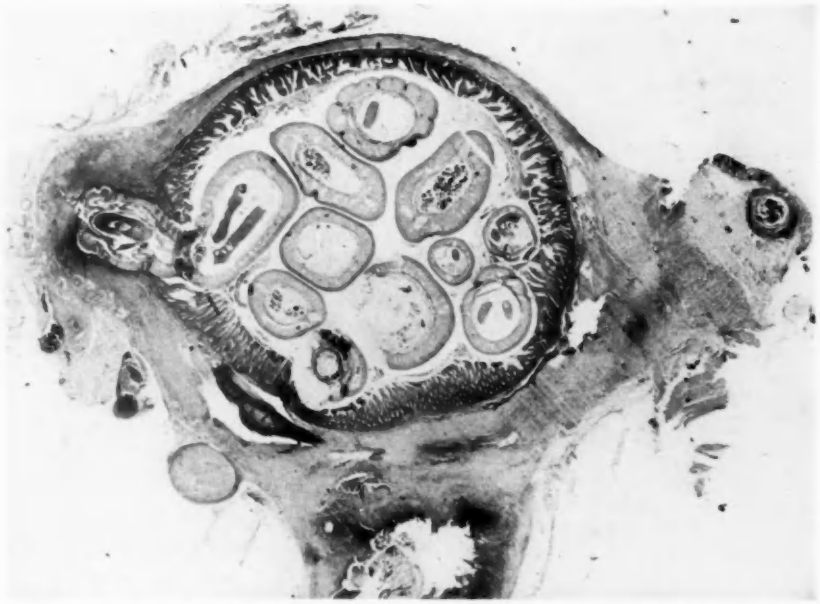

FIG. 1. Low power cross-section of marmoset colon, showing large numbers of *Prosthenorchis elegans* present, and type of tissue reaction at sites of attachment of the acenthocephalid parasites. 10.4 ×, hematoxylin-eosin.

FIG. 2. Enlargement of portion of colon showing penetration of the bowel wall and type of tissue reaction to the parasite. 42 ×, hematoxylin-eosin.

FIG. 3. Cross-section of *Prosthenorchis* in tunnel in the marmoset colon. Note lack of tissue reaction to the parasite. 42 ×, hematoxylin-eosin.

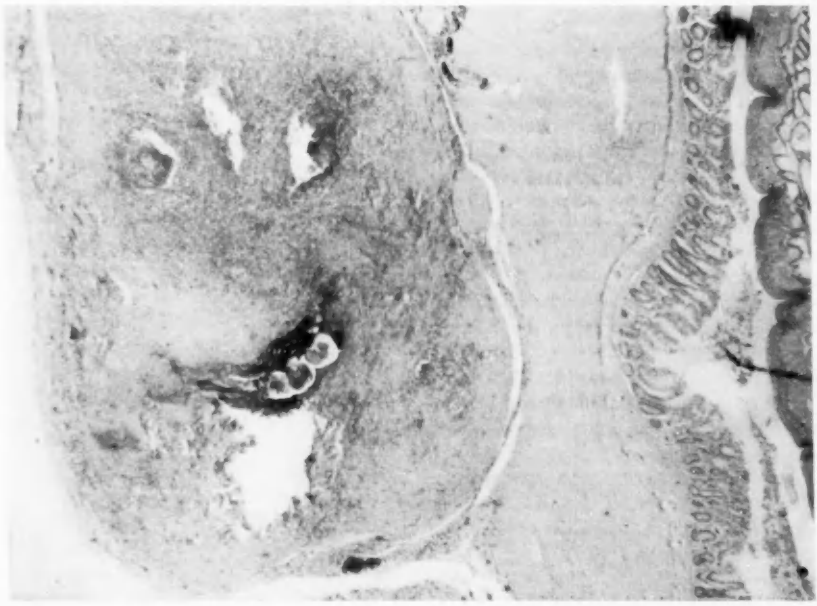

FIG. 4. Enlargement of nodule on peritoneal surface of colon formed in response to the attachment of *Prosthenorchis*. Note fragments of cuticle, showing worm has perforated into the nodule, and the dense infiltration by neutrophils indicating early abscess formation. 42 ×, hematoxylin-eosin.

Usually there was no evidence of tissue reaction to the presence of the parasites, and the majority of the tunnel walls were completely free of cellular infiltration (Fig. 3). The sections cut from those areas where perforation had occurred presented a different appearance. Here, dense accumulations of polynuclear leukocytes surrounded the tunnels and extended into the nodules on the serosa of the bowel. Sheets of this acute inflammatory exudate extended into and through the muscle coat of the colon, destroying the normal architecture of the bowel wall. Practically unbroken masses of these cells extended from the mucosa to the serosal surface of the bowel (Fig. 4). Scattered masses of bacteria were identifiable in the cellular exudate.

The nodules of the serosa were covered by visceral peritoneum and their substance was formed of dense interlacing strands of eosin-staining material having the appearance of fibrin. The peritoneum covering the nodules was sometimes thickened and infiltrated by scattered collections of mononuclear cells resembling lymphocytes. No such evidence of chronic inflammation was found in the adjacent tunnel areas. The serosa of the areas involved in the acute peritonitis was covered by masses of cellular exudate made up almost entirely of polymorphonuclear leukocytes.

Blood cultures were made at necropsy with heart's blood removed sterilely from the animals. Three of the four marmosets had a septicemia, and pure colonies of a Gram-negative, motile organism similar to the colon bacillus were recovered. Bacterial cultures run on the inflammatory exudate yielded a mixture of organisms, chiefly of the colon-aerogenes group. No cause of death other than peritonitis was found in the *Prosthenorchis* infected animals. The two experimental monkeys, A-14 and A-18, did not have lesions of cryptococcosis, but the yeasts were recovered from cultures made from the lungs.

Marmoset A-18 was found to have a grossly distended small intestine at autopsy. The ileocecal valve of this animal was completely obstructed by a large number of Acanthocephala which were apparently migrating from their usual habitat in the large intestine.

DISCUSSION

The present studies indicate that *Prosthenorchis* produces mechanical damage and it is only when bacteria gain access to the submucosal tissues, probably through tunnels, that perforation takes place. The phlegmon was associated with those areas of the bowel wall where perforation had taken place. Consequently, secondary infection of the tissues by bacteria from the bowel was probably directly responsible for both the perforation and peritonitis.

Fatal peritonitis not infrequently accompanies infections by Acanthocephala of the domestic pig. A study of such lesions by Weinberg and Romanovitch (1907) concluded that the spiny-headed worms were responsible only for mechanical damage and the actual process of perforation resulted from acute bacterial infection of the lesions produced by the worms. Vernard and Warfel (1947) described mechanical damage to the gut of the large-mouthed black bass caused by two species of Acanthocephala.

Acanthiasis epidemics among confined primates causing deaths due to Acanthocephala of the genus *Prosthenorchis* have occurred in laboratories both in the United States and in Europe. Rateliffe (1930), Van Cleave (1953), Brumpt and Urbain (1938), Van Thiel and Wiegand-Bruss (1945) and Chandler (1953) all attest to this fact. Brumpt and Desportes (1938) demonstrated that the intermediate hosts are cockroaches, as did Chandler (1953). This last author also has shown that cockroaches harbor the acanthellas which infect when swallowed by the final hosts. He also gives the first report of a natural infection with this parasite in the wild coati in Chiapas, Mexico. Heretofore it was considered endemic only in Central and South America. Van Cleave (1953) in a survey of the literature on on *Prosthenorchis elegans* makes it clear that in the New World this is the only species infecting primates. It is pathogenic and frequently lethal to its host.

There can be little doubt that the squirrel marmosets of the present study were infected with *Prosthenorchis* before arriving at the laboratory. These parasites were not seen in other monkeys obtained from the same source, and had never been found in a large number of the common marmoset, *Leontocebus geoffroyi*, which was frequently used at the laboratory. Considering the numerous tunnels opening into the lumen of the colon, it was remarkable that more bacterial infections did not occur soon after infection by *Prosthenorchis*. It is possible that the bodies of the worms within the tunnels may prevent the entrance of bacteria in most instances. Possibly the worms secrete some sort of bacteriostatic substance which keeps the exposed tissues free of bacteria.

SUMMARY

Four of ten squirrel-marmosets died as a result of infection by an acanthocephalid parasite, *Prosthenorchis elegans*. Death followed peritonitis caused by perforation of the bowel. Evidence is presented indicating that the process of perforation was a direct result of secondary bacterial infection rather than of the action of the acanthocephalid parasites. These spiny-headed worms produced chiefly mechanical damage.

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A STUDY OF PROTHROMBIN TIME AND THROMBOTIC PHENOMENA IN CHOLERA

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A peculiar phenomenon which is commonly observed in cases of cholera, is the quick clotting of blood in the syringe during the taking of samples for routine laboratory examinations. In fact, clotting occurs so rapidly that the blood often coagulates within the syringe even before 5 or 6 cc. have been withdrawn. Consequently, the difficulty of obtaining samples has often to be overcome by two workers with different syringes which can be fitted to the same identical needle. When 5 cc. of blood has been withdrawn in one syringe, it is immediately removed leaving the needle in situ and the contents delivered into a suitable test tube, while the other worker fits the second syringe to the needle and completes the job (Chatterjee, 1946).

To throw light on this phenomenon of increased coagulability as well as to find an explanation of the various thrombotic phenomena which are seen in cholera, we have studied the prothrombin time of blood together with its specific gravity and corpuscular volume.

METHODS

The method adopted in our cases has been a modification of Quick's technique (Quick *et al.*, 1935; Quick, 1938) as adapted by Fullerton (1940) using Russell's viper venom as the source of thrombokinase. The Russell's viper venom (Vipero-Toxin) was obtained locally. It was diluted with double distilled water to make a solution of 1/10,000. This solution was titrated against freshly drawn normal plasma. By trial and error, a solution was found which gave with normal plasma a clotting time of about 25 seconds. For our batches of venom, the appropriate dilution is in the neighborhood of 1/20,000. This diluted venom is stored in the refrigerator.

The blood is withdrawn by venipuncture and mixed with M/10 (1.34%) sodium oxalate solution in the proportion of 9 parts of blood to 1 part of oxalate solution. It is centrifuged and the clear plasma separated.

In a small (6 x 1 cm.) test tube 1.2 cc. of the plasma and 0.2 cc. of the thromboplastin solution are mixed. This is placed in a water bath at 37°C. After 1 to 2 minutes 0.2 cc of M/40 calcium chloride (pure anhydrous 1.11% soln.) already warmed to 37°C. is rapidly added and the stop watch is started. The contents are allowed to mix for 4 to 5 seconds. The tube is again placed in the water bath and the time noted when the first strands of fibrin appear (shown by gently inverting the tube). This is taken as the prothrombin time. The estimation is done three times and the mean value of these observations gives the mean prothrombin time. All glassware must be dry and chemically clean. When the

thromboplastin solution gives with normal plasma a clotting time other than 25 seconds, the following correction factor is applied.

$$\text{Corrected clotting time} = \frac{25 \times U}{N}$$

where U is the unknown plasma clotting time and N is that of normal plasma.

Fifty eight cases of clinical cholera have been studied, the first blood specimens being collected on admission before administration of saline or any other treatment. Of these, 42 cases showed cholera vibrios on culture of stools (Chatterjee, 1953, 1956; Chatterjee *et al.*, 1955a), and only these culture-positive cases have been taken into consideration in the present study. The observations were repeated during the course of treatment with saline etc. and also during convalescence when the normal specific gravity of the blood had been restored along with clinical improvement. In a few cases similar observations were made although clinical improvement was not apparent.

OBSERVATIONS AND DISCUSSION

We studied as a rule only fairly severe or very severe cases where the initial specific gravity of the blood was 1.064 or more before transfusion. The normal mean standards for blood are: specific gravity 1.056; corpuscular volume 40 per cent; and prothrombin time 25 seconds (Basu and Chatterjee, 1934; 1937). In general our cases showed an increase in corpuscular volume, more or less in direct proportion to the rise in specific gravity, and a marked decrease in prothrombin time, although this was not in strict correspondence with the rise in specific gravity (Table 1).

With the restoration of normal specific gravity and general clinical improvement, samples of blood were re-examined 72 to 96 hours after admission. As might be expected there was a lowering of average corpuscular volume and an increase in the prothrombin time (Table 2). In four very severe cases, in spite of restoration of normal specific gravity, clinical improvement was not noticeable. A notable feature in these cases was the marked dyspnea with hurried respiration (Table 3).

This study shows that there is a great tendency for the prothrombin time to

TABLE 1

Specific gravity, corpuscular volume, prothrombin time of blood and mortality in cholera cases

No. of cases	Blood sp. gr.	Corpuscular volume %			Prothrombin time (secs.)			Mortality	
		Min.	Max.	Avg.	Min.	Max.	Avg.	Number	Per cent
7	1.064	40	53	45.6	14.2	20.3	17.9	Nil.	
7	1.066	40	60	52.5	12.5	20.1	15.1	Nil.	
8	1.068	40	65	57	11.8	18	14.5	1	12.5
14	1.070	50	70	58.4	11.8	18.3	15.2	2	14.2
6	1.072	55	70	63	12.5	15.8	14.9	1	16.6

TABLE 2

Improvement in blood of convalescent cholera cases after treatment

Number of cases	Specific gravity	Corpuscular volume			Prothrombin time		
		Min.	Max.	Avg.	Min.	Max.	Avg.
10	1.056	35	45	40	21.9	29.7	23.2
17	1.058	33.3	50	42.4	20.3	25.4	23.6

TABLE 3

Findings in cases with great dyspnea after the restoration of normal specific gravity of blood

Case No.	Specific gravity of blood	Prothrombin time	Results
28	1.058	15.8	Died
34	1.060	16.9	Prolonged convalescence
36	1.058	18.3	Prolonged convalescence
46	1.058	16.8	Prolonged convalescence

decrease in cholera. In this connection it is interesting to recall the work of Lahiri (1935) in which a shortening of the coagulation time of blood in cholera patients was observed. This was found to be somewhat independent of the specific gravity of the blood and seemed to depend more on the severity of the case.

THROMBOTIC COMPLICATIONS OF CHOLERA

Femoral thrombosis. Two cases of femoral thrombosis occurred in our series of 1,800 admissions for cholera during the 1955 epidemic.

Cerebral thrombosis. Four cases of hemiplegia and one of bilateral paralysis were observed in the same series, diagnosed as due to cerebral thrombosis. All were pulseless on admission, and when the blood pressure could be taken it was fairly low, lying between 110–140 mm. systolic, and 80–90 diastolic. The history was the typical one of an initial feeling of weakness in the legs gradually extending upwards until paralysis was complete within 72 hours. The relatively slow advance of the paralysis suggested thrombosis rather than hemorrhage, a conclusion which was confirmed clinically by the partial recovery of 4 of the cases. There was some sensory disturbance in one case. The ophthalmoscope showed arterial changes in 4 of the 5 cases.

The four cases of hemiplegia (2 male and 2 female, aged 43, 44, 55 and 62) were eventually discharged from the hospital with partial restoration of movement. A followup of these individuals was not possible owing to the great pressure of work during the epidemic. In the fifth case (a male, 45 years old) the paralysis extended to both sides of the body and resulted in death.

Pulmonary embolism. In 1954 we saw two fatal cases which showed the typical textbook picture of pulmonary embolism with dyspnea, pain in the chest, shock and hemorrhagic sputum. During transfusions, a very large percentage of cases developed dyspnea which might have been due to various causes. It has been

remarked that only one-third of cases of pulmonary embolism can be diagnosed during life (Landet, 1955). Considering the number of cases showing great dyspnea in spite of the restoration of blood volume and specific gravity, it is not unlikely that many of these cases might have been diagnosed cases of pulmonary embolism, especially when uremia and kidney failure can be excluded.

Thrombophlebitis. In the majority of serious cases of cholera, repeated intravenous transfusions have to be given and consequently the leg veins are used. Moreover, for the purpose of the drip method of transfusion the inferior extremity is more suitable as it gives better facilities for fixation, particularly in restless patients. This would undoubtedly lead to increased chances of thrombophlebitis of the veins, especially in the middle-aged and the elderly cases, as has been clinically described (Chatterjee *et al.*, 1955b).

It is now realized that pulmonary embolism is much commoner than was formerly supposed (Lancet, 1955). Necropsy figures published by Moran (1947) and by Towbin (1954) give an idea of the frequency of this condition which is often overlooked or mistaken during life. In Moran's cases (1947), the incidence of pulmonary embolism was 23.1 per cent in a series of 635 autopsies in adults and in Towbin's cases the figure was 25.7 per cent in a series of 512 autopsied in adults.

CHOLERA COMPLICATING PREGNANCY

The phenomenon of increased coaguability of blood seems to be of some importance in cases of cholera occurring during pregnancy. We have found that in these cases also there is markedly reduced prothrombin time, averaging 14.5 seconds in 14 cases. Possibly this is not an unmixed evil as there seems to be a greatly reduced *post partum* hemorrhage which rarely extends beyond the first day.

Regarding the prognosis of the mother, it does not appear to be in any way worse than that of the average case of cholera. Thus in a series of fourteen cases (thirteen multiparae and one primipara) who were attacked with cholera, there was only one fatality (in a multipara), the mortality rate being 7.2 per cent as compared to 7.0 per cent mortality of all cases of cholera in our ward in 1955. The period of gestation varied in the above cases from five months to full term, pregnancy being terminated by the attack of cholera.

Regarding the prognosis of the child, in thirteen out of fourteen cases the fetus was still-born and in the remaining case the child died after three days.

As a matter of interest, fourteen cases of normal pregnancy with gestation from eight months to full term were also examined for prothrombin time. The prothrombin time was found to be reduced below 20 seconds in eleven (64%) of the cases and was normal in the rest (36%).

SUMMARY

Forty-two cases of cholera were examined for prothrombin time before administration of transfusion as well as after treatment. A definite decrease in prothrombin time was observed, which tended to become normal during con-

valescence. In some clinically severe cases, even after restoration of the blood to normal specific gravity, the prothrombin time remained low. The importance of the study of prothrombin and its relation to the various thrombotic phenomena that occur in cholera is evident.

In all cases of cholera superimposed on pregnancy, as in some cases of normal pregnancy, the prothrombin time was found to be reduced. In cholera cases this seems to be not an unmixed evil as there is a greatly reduced *post partum* hemorrhage which seldom extends beyond the first day.

ACKNOWLEDGEMENT

We offer our thanks to the authorities of Chittaranjan Hospital for their co-operation.

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PULMONARY ASCARIASIS RESEMBLING EOSINOPHILIC LUNG

Autopsy Report with Description of Larvae in the Bronchioles[1]

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Reviews and reports on eosinophilic lung (tropical eosinophilia) have stressed two generalizations. First, while there is a wide range in the symptoms and clinical findings associated with high eosinophilia, there is recognizable among such cases a group which seems to represent a single disease entity in which the prominent and more or less constant characteristics are extreme eosinophilia and asthma-like symptoms, both of which respond to treatment with organic arsenicals given intravenously or orally. The second feature, constantly emphasized, is the lack of a demonstrated etiologic agent (Ball, 1950; Danaraj, 1951; Crofton *et al.*, 1952; Aitken and Roy, 1953; Chaudhuri *et al.*, 1954; Diaz-Rivera *et al.*, 1954; Coutinho, 1956).

The present report is based on observations before and after death of a resident of Singapore, who, after a brief illness, died without having received specific treatment. A provisional diagnosis of eosinophilic lung was made in view of the pulmonary symptoms and hypereosinophilia in an adult Indian. However, the period of observation was short, essentially limiting the investigations to a brief study of the blood. Nematode larvae, found in the bronchioles and identified as *Ascaris* sp., appear to have been chiefly responsible for the disease.

CASE REPORT

An adult male Indian, resident in Singapore, was admitted to the General Hospital presenting a typical picture of status asthmaticus which had become increasingly severe for the past ten days. On the basis of the clinical history, findings on physical examination, and an eosinophilia of 37 per cent of 21,400 white blood cells, a diagnosis of eosinophilic lung was made. Although efforts to relieve the bronchial spasm were partly successful he died the following day. Post-mortem examination revealed massive infiltration of the lungs with eosinophilic leucocytes and changes compatible with bronchial asthma. Scattered linear, eosinophilic, granulomatous lesions were found in the liver. In sections of lung tissue, five nematode larvae were found, each in a bronchiole that was entirely filled with mucopurulent exudate.

Hospital record. A. K., a male Tamil Indian laborer, aged 30 years, was admitted to hospital on 9 January and died 10 January 1951. He was well and working until 10 days before admission when he developed paroxysms of cough,

[1] An investigation conducted under the sponsorship of the Commission on Parasitic Diseases, Armed Forces Epidemiological Board, and supported in part by the Office of the Surgeon General, Department of the Army.

worse at night, sufficiently severe to prevent his going to work. There was pain over the front of the chest. A slight amount of whitish sputum was produced at the end of each paroxysm, but no blood. He had no fever. After the sixth day of illness, breathlessness became severe and continuous, with hardly any coughing. He had to sit up in bed sweating, could not sleep and became exhausted; his eyes were sunken. A general practitioner gave him some injections without relief. On examination: temperature 98°F.; pulse rate 130; respiratory rate 48 per minute; sitting up in bed in considerable distress. Accessory muscles of respiration very prominent. Pouring sweat over face, chest and arms. Audible expiratory wheeze. Chest hyperresonant, heart sounds hardly heard, expiration considerably prolonged; wheezing ronchi heard all over. Spleen and liver not felt. No abnormality of the central nervous system was detected. Laboratory findings: Blood film negative for malaria parasites. Red blood cells, 5.3 million. Hemoglobin 110 per cent. White blood cells 21,400: neutrophils 55, lymphocytes 6, monocytes 2, eosinophils 37 per cent.

There was slight relief after use of oxygen and various drugs (aminophylline intravenously, penicillin, ephedrine sulphate, adrenalin, paraldehyde, soneryl, and an antispasmodic mixture), but at no time was the spasm completely relieved. On the following day the temperature was 100, pulse rate 128, and respiratory rate 56. He continued to look exhausted with sunken eyes. When examined at 4:00 P.M. (10 Jan.) he was sitting propped up in bed breathing rapidly and audibly. There was prolonged expiration with scattered wheezing ronchi and crepitations in both bases. Bronchial spasm, however, did not appear as severe as on the preceding day. Heart sounds were obscured by the ronchi. The cervical veins were not engorged and the liver was not palpable. There was definite twitching of the upper lip on tapping the facial nerve. He was given an injection of calcium gluconate intravenously after which he appeared to go to sleep. He was found dead at 6:40 P.M.

Necropsy observations. (9:00 A.M. 11 Jan.). Body well-nourished male adult Indian. Pericardial sac contained 45 ml. serous fluid. There was slight hypertrophy of the right chamber of the heart with marked dilatation; left chamber normal. Larynx and trachea normal. Bronchi near the hilum of both lungs showed congestion and granularity of mucosa, some filled with thick, tenacious yellowish exudate. Cut surfaces of the lungs showed irregular reddish grey patches with induration; some were friable on deep pressure. The entire lung was voluminous and diffusely emphysematous with a few bullae over the apices. Tracheo-bronchial glands were enlarged. Liver slightly swollen with purplish patches beneath the stretched capsule, and the centers of some of them showed yellowish grey nodules. The cut surface showed early nutmeg appearance. Wall of gall bladder slightly edematous, not typical of beri-beri. Kidneys congested. Spleen, suprarenals, pancreas, stomach and intestines normal. Brain not examined.

Histo-pathological report. Liver—Selected slides containing sections through gross lesions in two different blocks all show changes that are essentially similar, consisting of extensive congestion and necrosis of the central areas with preserva-

tion of the periportal zone. There is diffuse pigmentation, particularly in the necrotic areas and some of the pigment appears to be within Küpffer cells. However, much of the pigment may be artefacts of fixation. In zones up to 2 mm. by 4 mm. in size, there are scattered, more or less confluent granulomatous lesions consisting of central areas of necrosis surrounded by a prominent zone of eosinophilic and neutrophilic leucocytes beyond which there is hepatic parenchyma showing variable degrees of degeneration. In some areas, there are epithelioid cells and in others giant cells of the foreign body type (Fig. 1 A). In none of the granulomatous lesions are parasites or any other specific agent seen. The portal spaces show prominent infiltration with polymorphonuclear leucocytes, including both neutrophils and eosinophils.

Lung—The sections available for study, some stained with hematoxylin and eosin, others with Giemsa, represent several blocks of pulmonary tissue in which the changes are essentially similar. The bronchi and bronchioles are dilated and contain mucopurulent material in which polymorphonuclear leucocytes of eosinophilic and neutrophilic varieties are prominently found. In addition, in some of the areas of cellular exudate where eosinophils predominate there are numerous Charcot-Leyden crystals (Fig. 1 B). In several of the bronchioles there are one or more portions of a nematode larva (Fig. 1 C). The surrounding pulmonary parenchyma shows several abnormalities. There are areas of partial atelectasis and other areas of inflammatory reaction characterized by thickening of the alveolar wall and infiltration with various types of leucocytes. In other areas, there is partial organization of the inflammatory exudate with the presence of fibroblasts within the alveoli. The peribronchial tissues show, in addition to inflammatory reaction, the presence of a prominent hyaline membrane in the subepithelial space (Fig. 1 D). In the subpleural areas, there are collections of anthracotic pigment, but elsewhere there is other granular pigment, morphologically resembling hemosiderin. The sections of pulmonary artery included show minimal intimal sclerosis. In some areas, the pulmonary parenchyma is poorly preserved, showing extensive autolysis. Histologic diagnosis: (1) Chronic passive congestion of liver; (2) central necrosis of liver; (3) granulomatous lesions of liver; (4) chronic bronchitis, due to larvae of a nematode; (5) bronchiectasis; (6) chronic pneumonitis; (7) changes compatible with chronic bronchial asthma.

Comment

On the basis of a study of 150 cases seen in Singapore, Danaraj (1951) characterized typical eosinophilic lung as a chronic condition in which the chief complaint is cough associated with breathlessness, worse at night, varying in degree from mild cough to status asthmaticus of great severity. Response to symptomatic treatment with antispasmodics is not as prompt nor as complete as in bronchial asthma. Eosinophilic leucocytosis, raised erythrocyte sedimentation rate without fever, and pulmonary shadows are prominent features. There is no enlargement of the spleen, lymph nodes or liver. A striking characteristic is prompt clinical improvement (occasionally after exacerbation initially) with a

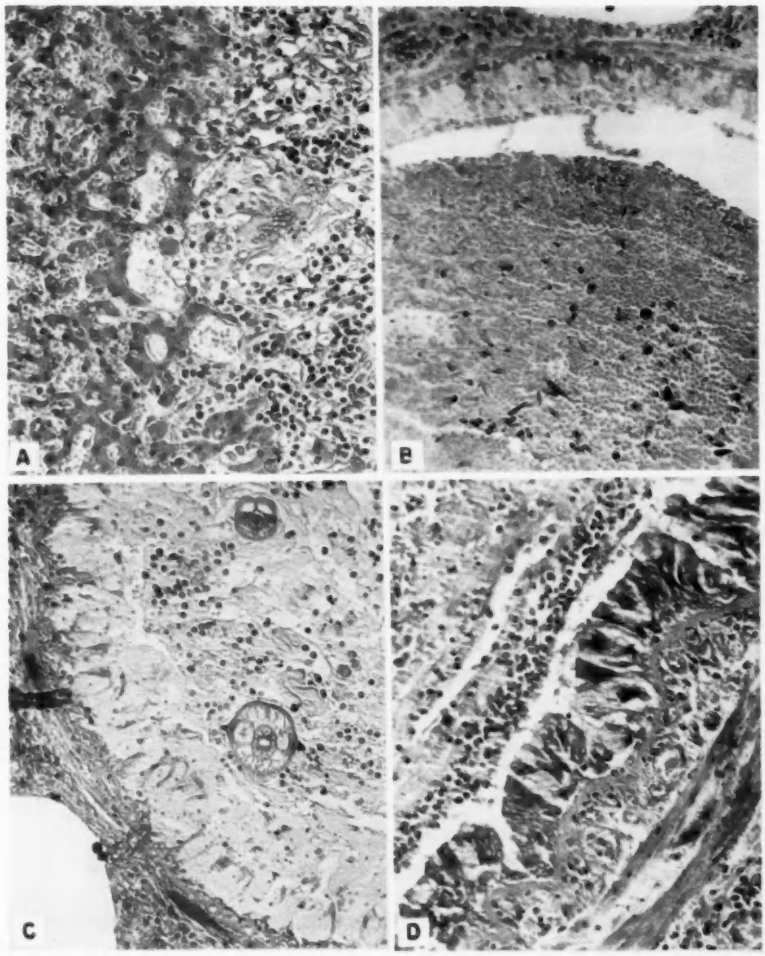

FIG. 1. Human autopsy sections showing some characteristic histo-pathological features.

A. Margin of granulomatous area of liver containing a giant cell and abundant eosinophils. × 170

B. Bronchiole filled with eosinophils and numerous Charcot-Leyden crystals cut at various angles. × 135

C. Bronchiole entirely filled with mucopurulent material containing a larva cut transversely through the esophageal and mid-intestinal regions. × 170

D. Wall of bronchiole with prominent hyaline membrane in the subepithelial space with abundant eosinophils in the lumen and surrounding the muscularis. × 170

fall in the eosinophil count resulting from treatment with organic arsenicals, oral or intravenous, and eventual cure. More recently, equally striking results have been obtained from oral administration of diethylcarbamazine (Danaraj, 1956).

The salient clinical features that differentiate eosinophilic lung from other respiratory disorders associated with an eosinophilia are: (1) a massive eosinophilic leucocytosis of over 3,000 eosinophils per cu. mm, (2) pulmonary shadows in the roentgenograms, (3) a raised erythrocyte sedimentation rate and (4) a therapeutic response to organic arsenicals. The latter feature clearly separates eosinophilic lung from other conditions with eosinophilia.

Differential histo-pathological features of eosinophilic lung are essentially unknown. In two cases in which death was due to arsenical encephalopathy following treatment of eosinophilic lung, the pertinent lesions in the lungs were primarily bronchial and peribronchial with eosinophilic leucocytes predominating among the infiltrative and exudative elements (Viswanathan, 1947; Danaraj, 1951). However, in both instances the original histo-pathological picture probably was considerably altered by the drug, directly and by the fatal reaction to it. These being as far as is known the only reported autopsy observations of eosinophilic lung, the gross and microscopic features of the pulmonary tissues can be inferred only from the characteristic symptoms and clinical findings. Hepatic tissues observed in biopsies from an individual with infiltrative eosinophilia which responded to arsenotherapy (Diaz-Rivera *et al.*, 1954) and with tropical eosinophilia (Chaudhuri, 1956) show marked eosinophilic infiltration along the portal tracts and abundant eosinophils in the sinusoids. This is a common finding also in visceral larva migrans, although the eosinophilic granulomatous type of lesion such as was seen in the present case is a more characteristic and more conspicuous feature seen in liver biopsies in this disease (Beaver *et al.*, 1952; Milburn and Ernst, 1953). Characteristic lesions described in autopsy reports of visceral larva migrans (larval toxocariasis) have thus far largely been limited to reactions around or in the paths of the larvae found in the liver, lungs, brain and other organs (Dent *et al.*, 1956), except in one case in which there were disseminated lesions, often in relation to severely altered small veins, along with extensive degeneration and some inflammation in the voluntary muscles and in the diaphragm (Brill *et al.*, 1953).

The histo-pathology of larval ascariasis in human tissues likewise has not been described. Much, however, can be inferred from studies on experimental animals and from symptoms, roentgenograms and the elements found in the sputum. From three experimental infections in human volunteers (Koino, 1922, 1923; Vogel and Minning, 1942; Essellier and Koszewski, 1951), among others, in which massive numbers of eosinophils and Charcot-Leyden crystals were observed in the sputum, along with signs and symptoms of bronchial pneumonia and bronchial spasm, it may be judged that after about a week following ingestion of infective eggs, there follows a period of several days during which the histopathological picture, if observable, would include chiefly hemorrhage into the alveoli and respiratory bronchioles, inflammation of the bronchial and peribronchial tissues, and heavy mucopurulent exudation into the bronchioles where, owing to the massive accumulation of eosinophilic leucocytes, Charcot-Leyden

crystals would be found, in some areas abundantly, along with other features such as emphysema, bronchiectasis and scattered areas of pneumonitis. Hemorrhage, not observed in the present case, is a prominent feature of larval ascariasis in swine and experimental animals during the eighth to tenth days of infection, at which time most of the migration of larvae from the parenchyma to the bronchi takes place. It is not possible at the present time accurately to interpret those changes in the tissues which indicate greater chronicity than can logically be expected from migration of the observed larvae.

It is significant that most of the reports of eosinophilic lung, variously designated, have come from India. In Singapore where the population is approximately 7 per cent Indian[2] and 78 per cent Chinese, and the hospital records show that 3 times as many Chinese as Indians are treated for various conditions, about 80 per cent of the eosinophilic lung cases occur among Indians (Danaraj, 1951). So that in any Indian with pulmonary symptoms and hypereosinophilia, a diagnosis of eosinophilic lung must be considered. In the present case, certain criteria such as chronicity of symptoms, roentgenographic shadows, raised E.S.R. and therapeutic response to organic arsenicals were not fulfilled, and the diagnosis is at the most only tentative. Had this patient survived, and observation continued, it is quite likely, in view of the size and location of the *Ascaris* larvae found in the lungs, that the symptoms would have cleared with a concomitant fall in the eosinophil count. Such "spontaneous cures" have been noted in a few cases diagnosed as eosinophilic lung in Singapore and hence it has been the practice to commence arsenotherapy only after a period of preliminary observation if the symptoms and hypereosinophilia are persistent. It is probable that some of these cases with "spontaneous cures" are really cases of *Ascaris* pneumonia in hypersensitive individuals.

THE LARVA

Sections were prepared from lung, liver, heart and kidney. Larvae were found in the lung only. However, the liver sections contained granulomatous lesions suggestive of larval invasion and migration (Fig. 1 A). Five larvae were contained in 570 sections of selected blocks of lung, representing approximately 250 cubic mm of tissue. One was found in a block that was skip-sectioned, with the mounted sections containing portions cut transversely from the mouth, esophagus, mid- and hind-body regions. One was sectioned serially but included only the posterior fourth of the body. The others were complete in serial sections cut at various angles but only one, apparently dead before the tissues were fixed, was sufficiently straight to permit reasonably accurate longitudinal measurements. All of the larvae were found in bronchioles 0.6 mm. to 3 mm. in diameter; 2 were in the same bronchiole at slightly different levels. The larvae were all about the same size and apparently represent the early fourth stage of a single species of an ascarid similar to or identical with *Ascaris lumbricoides*.

Description. Length at least 1.8 mm., possibly more than 2.0 mm.; greatest

[2] Indian as used in this paper is construed to mean a member of any of the various races inhabitant in modern times of the Indian subcontinent, Ceylon, and East and West Pakistan and includes both immigrants from these places and their offspring born in Singapore.

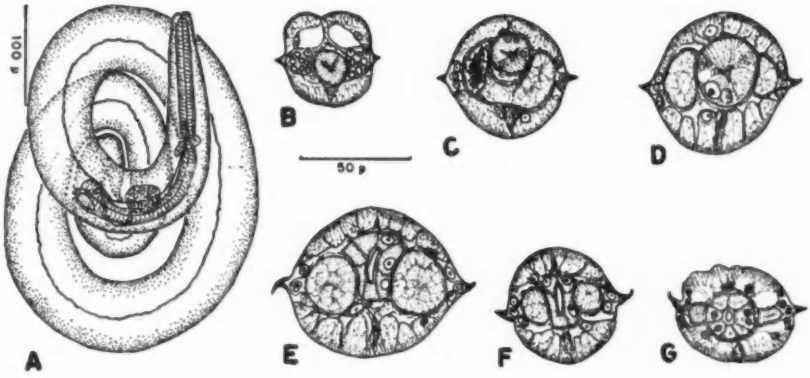

FIG. 2.

A. Model of larva found in human bronchiole, reconstructed from serial sections; esophagus, nerve ring and excretory-cell nucleus added from graphic reconstruction.
B through G are camera lucida drawings of transverse sections of larvae, equally magnified.
B. Larva in human lung, cut at level of ganglionic mass, just posterior to the nerve ring.
C. Same, cut through esophageal region at level of excretory-cell nucleus and commissure.
D. Same, through level of esophageal bulb. Excretory columns and lateral chord cells lie between it and the lateral alae.
E. Same, at level of mid-intestine where excretory columns and lateral alae are most prominent.
F. *Ascaris lumbricoides* larva in bronchiole of pig, 8 days after inoculation, cut at level of mid-intestine. It differs essentially from E only in its size and number of intestinal cells.
G. *Ascaris columnaris* larva in heart muscle of mouse, 16 days after inoculation, cut at level corresponding with E and F. Intestinal cells are numerous and excretory columns are relatively small.

width just back of esophageal bulb; dorsoventral diameter slightly less than transverse, the latter being approximately (exclusive of alae) 24 μ at anterior tip of esophagus, 50 μ at nerve ring, 65 μ at esophageal bulb, 70–75 μ at middle portion and 32 μ at rectum. The tip of the tail is flexed dorsally. Cuticle transversely striated with annulations about 2 μ wide. Lateral alae extend from in front of nerve ring to just back of cloaca, 7–10 μ high from level of esophageal bulb to mid-body, apparently flexible since tips curve ventrally or dorsally in different sections from the same regions and on the two sides of the same sections. They tend to be hollow at the base (Figs. 1 C, 2 A–E). Muscle cells of body 3 or 4 in each quadrant, heavier on the ventral side; subventral muscle bands clearly set apart by the ventral chord; dorsal chord inconspicuous in some sections. Lateral chords contain three distinct rows of nuclei placed about 4 per 25-micron interval (Fig. 2 C, D, E). Esophagus approximately 0.285 mm. long, with its diameter 28 microns at the bulb, 15–16 at the nerve ring and about 18 at other levels. The nerve ring is approximately 0.12 mm. from the mouth. Immediately behind it is a basophilic ganglionic mass mostly lateral and ventral to the esophagus (Fig. 2 B). Overlapping the ganglionic mass are the lateral excretory-cell

columns which extend only a few microns forward from the level of an extremely large, single excretory-cell nucleus whose dimensions are approximately 10 by 22 by 28 microns (longest antero-posteriorly) and is located on the left side of the esophagus just anterior to the bulb (Fig. 2 C). At its level a wide commissure joins the right and left sides of the excretory cell ventral to the esophagus. Large spongy (non-tubular) excretory columns extend along the lateral chords posteriorly from the excretory cell nearly to the cloaca. The left column is larger and somewhat longer than the right (Fig. 2 D, E). Here and there near the center of the columns the spongy material is dense, suggesting a collapsed tubule but there apparently is no continuous central canal. The maximum diameter, measured transversely, is 25 μ; dorsoventrally, 30 μ. The intestine is well developed with 7 or 8 cells in each transverse section and is distinctly compressed between the excretory columns at most levels. Its lumen is lined with hyaline material and does not contain particulate matter in any of the 5 specimens (Fig. 2 E). The anus is 70 μ or more from the tip of the tail. Genital primordia and details of the oral structures were not evident in any of the specimens.

Comment

On the basis of detailed descriptions of the larvae of *Ascaris lumbricoides*, *Toxocara canis* and several other species given by Nichols (1956 a, b), the larva described above can readily be identified as belonging to the same group as *Ascaris lumbricoides*. It definitely differs from all other described species and is in nearly every respect identical with the early fourth-stage larva of *A. lumbricoides*. No essential difference can be detected in the body proportions, lateral alae, excretory system, digestive system, lateral chords, nervous system or body wall (Fig. 2 E, F). The largest fourth-stage larvae of *A. lumbricoides* yet described in detail is much smaller than those found in the present case, the former being only 1.6 to 1.7 mm. long by 54 μ in maximum transverse diameter while the latter is somewhat longer and is more than 70 μ in diameter. However, Nichols recovered free larvae from the lungs of mice up to 2.0 mm. in length and Roberts (1934) found larvae up to 2.4 mm. long in the lungs of pigs. Widths were not recorded for these larvae but it is probable that where the length is over 2.0 mm. the width may be 70 μ or more. Koino (1922, 1923) collected numerous larvae (178 in one day) from his sputum 8 to 16 days after swallowing infective eggs and recorded 1.746 by 0.099 mm. as the average length-width measurements but the width measurement is questionable. During growth of the larvae, morphological changes are most noticeable in the number of cells in the intestinal wall. In larvae with diameters around 50 μ, usually 4 or 5 cells can be seen in transverse sections (Fig. 2 F). In larvae of *A. columnaris* of similar size, the number of intestinal cells seen in transverse sections is 7 or 8 (Fig. 2 G). Also in this species the excretory columns are smaller and the cells of the lateral chords are more numerous. It is evident from this type of comparison that the larva found in the human lung probably is *Ascaris lumbricoides*, but until it can be compared with a larva of equal size and known to be *A. lumbricoides* there must remain some question about its identification.

DISCUSSION

Omitting the question of the direct or primary cause of death which in the case under discussion is secondary to other considerations, and granting that the observed symptoms and pathology were related, and granting further that the larvae found in the bronchioles are *Ascaris lumbricoides*, a question that still must be considered is whether the presence of the larvae was the primary cause of the symptoms and pathological conditions.

It is well established by experimental infections of *Ascaris lumbricoides* in man, pigs and smaller laboratory hosts that the larvae on hatching in the small intestine migrate through the liver to the lungs, where on the eighth or ninth day after inoculation they begin migrating further into the bronchial passages and, via the trachea and esophagus, return to the intestine. Growth occurs in the parenchyma of the liver and lungs, and when the larvae enter the bronchioles they ordinarily have progressed from the second stage (infective), 0.3 mm. or less in length, to the early fourth stage which is 1.5 mm. or more in length. Considering in the present case, that the onset of symptoms occurred ten days prior to death, that there was evidence of earlier heavy migration through the liver, and that the larvae apparently were abundant only in the bronchioles, there would seem to be strong indication of a direct relationship between the larval infection and the observed disease.

Experimental infections with *Ascaris lumbricoides* in man and experimental animals have shown that the larvae in their migration and development in the liver and lungs, especially the latter, cause extreme eosinophilia of the blood and tissues along with cough and pulmonary infiltrations and other pathological changes that are roentgenographically evident. Such studies also have established the important fact that the severity of symptoms and tissue reaction is proportional to the size of the inoculum and the degree of sensitization resulting from previous exposure (Vogel and Minning, 1942; Sprent and Chen, 1949; Essellier and Koszewski, 1951). Light infections initially may cause no significant symptoms whereas a heavy infection such as the one reported by Koino (l.c.) or a light infection in a previously infected individual such as Vogel and Minning (l.c.) described (in which only 46 eggs were swallowed) may produce marked reactions. The symptoms resulting from such infections are transient, however, because *A. lumbricoides* larvae that fail to make the migration from the lungs or other tissues die within a few weeks and are destroyed, at least in experimental animals. This phenomenon and the symptom complex associated with it, Loeffler's syndrome, is observed commonly in some areas where ascariasis and similar helminthic infections are highly endemic. Presumably, if infections are acquired more or less continuously or in close succession, or are acquired in combination with infections producing the visceral larva migrans type of syndrome (Beaver, 1956), symptoms likewise would be continuous, perhaps with remissions and exacerbations at irregular intervals, producing in that manner a chronic condition that could not be recognized as Loeffler's syndrome but instead would resemble more the usual picture of eosinophilic lung. Experimental evidence in support of that possibility in man is lacking although repeated infections

in animals are strongly suggestive (Sprent and Chen, 1949). On the other hand, ascariasis as seen in swine would seem to indicate that under conditions producing continuous or frequent ingestion of infective eggs, tolerance and a high degree of immunity are developed and evident symptoms may not be produced. However, the ascariasis picture in swine is still confusing and may not be applicable to the situation in human populations. For example, while intestinal infection in swine is in general common, and migration of larvae through the tissues has often been observed to occur and to produce tissue damage in the same manner as in other animals, experimental infections that were known to have reached the intestinal phase in swine rarely have persisted to the egg-laying stage (Oldham and White, 1944). Similar experimental inoculations in man frequently have produced patent infections (Vogel and Minning, l.c.). Regardless of the origin of the infective eggs, i.e., from worms living in man or in swine, attempts to produce experimental infections apparently have been much more successful in humans than in swine (Takata, 1951; Yajima, 1955).

As regards the question of susceptibility to infection with *A. lumbricoides* and the damaging effects of the migrating larvae, individual and racial differences must be considered apart from the size or the sequence of infecting doses of larvae. Before pulmonary ascariasis can be accepted as a cause of eosinophilic lung in Singapore, its chronicity and response to arsenotherapy must be demonstrated and an explanation must be found for the observation that while *Ascaris*, hookworm and other sanitation-related intestinal parasites are equally common in Indians and Chinese, eosinophilic lung is many times more common in Indians than in other racial groups. Special allergy proneness and examples of individuals with extreme sensitization to *Ascaris* are commonly recognized. Recently, it has been noticed that in groups of children with more or less similar exposures to lung-invading helminth larvae the frequency of asthma, apparently caused by the larvae, varies widely in the different groups (Kempski, 1956). Also, it has been observed that in an area where Fijians and Indians are about equally infected with *Ascaris* and hookworm, 98 out of 100 cases of respiratory complications, including severe bronchospasm (one death), were among Indians (Hemming, 1956). These observations suggest that irrespective of specific allergens, asthmatic conditions are possibly more common among Indians than some other groups.

It would appear that as regards eosinophilic lung, the finding of *Ascaris* larvae in the case under discussion is of importance chiefly in that it has shown that in an area where eosinophilic lung is a common complaint among adult Indians, one of the other causes of hypereosinophilia with severe respiratory symptoms is larval ascariasis which, in view of the prevalence of intestinal ascariasis, may also be common.

CONCLUSIONS

In the case under discussion the nematode larvae discovered in the lung probably were directly responsible for the severe bronchial spasm, hypereosinophilia and most of the other conditions which preceded the individual's death. The

larvae can definitely be identified as early fourth-stage *Ascaris* and are probably *A. lumbricoides*. The relative importance of *A. lumbricoides* as an etiologic agent or complicating factor in eosinophilic lung cannot yet be determined but it seems probable that eosinophilic lung is a separate disease entity and that the case reported here was pulmonary ascariasis, the first in which the offending larva has been discovered and identified on the basis of its morphology.

SUMMARY

In the lungs of an adult Indian in Singapore who died after a ten-day illness which was tentatively diagnosed as eosinophilic lung, numerous nematode larvae were found. The larvae have been identified morphologically as *Ascaris* sp., probably *A. lumbricoides*. It is believed that the larvae were responsible for the disease observed in this case, and that pulmonary ascariasis and eosinophilic lung are separate but clinically similar disease entities.

The observations suggest that this and possibly other species of nematode larvae may cause pulmonary symptoms with hypereosinophilia resembling the condition described as eosinophilic lung, and in life the two conditions may be indistinguishable.

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ECOLOGICAL STUDIES OF WILD RODENT PLAGUE IN THE SAN FRANCISCO BAY AREA OF CALIFORNIA

I. Introduction

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HISTORICAL

There remain few arthropod-borne diseases in the United States which are of epidemic concern at the present time. This has been due largely to the rapid progress made in sanitation and in the improvement of living and working conditions for the major portion of the population. The rapid advances made in environmental control measures, the discovery and use of new insecticides, and strides in chemotherapeutic treatment and prophylaxis have also been of significant importance affecting the decline of these maladies. The arthropod-borne viral encephalitides seem to have usurped the central position on the stage of vector-transmitted diseases of man in North America.

This situation could easily lead to the entirely erroneous conclusion that we are permanently rid of such "classical" diseases as typhus, plague, yellow fever, and malaria. From the public health standpoint, it is important to emphasize that continued investigation, surveillance, and quarantine are necessary to maintain competent vigilance against possible outbreaks of these and other diseases.

Plague, like yellow fever, has its sylvan or wild phase, although it cannot be claimed that wild rodent plague in this country is now of major active concern to human populations. Nevertheless, there are substantial reasons for assuming that plague in the United States may be currently in a quiescent stage of unknown length since such inactive plateaus have been shown in this disease from knowledge of its epidemiology (Hirst, 1953). From 1900 to 1924 there were 10 epidemics of classical urban rat-borne plague in various port cities (Mohr, 1950), but no urban plague has been noted since that time. Thus it appears that rat-borne plague *per se* may be a thing of the past in this country.

Plague in the United States is now conceded to be firmly established in wild rodents and their fleas in the western region, as it is in western Canada. Very recently, plague was isolated from prairie dogs, *Cynomys mexicanus*, in the northern state of Coahuila, Mexico (Varela and Vasquez, 1954). The evidence shows that the disease in wild rodents forms a vast enzootic reservoir of paramount importance as a threatening source of infection to human beings (Pollitzer, 1954; Link 1955). In 1934 a sheepherder became ill with plague in Lake County, Oregon, and a search for infected rodents the following year in the same area revealed plague-infected ground squirrels. This was the first indication that wild rodents harbored plague in a state other than California. Extensive surveys by the Public Health Service and certain state agencies were conducted

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from 1936 to 1950 between the Pacific Coast and the 100th meridian in 17 western states (Wayson, 1947; Link, 1950). Wild rodent plague foci were found in 131 counties of 15 of these states. Thus it was shown that the plague organism, *Pasteurella pestis*, was established in an area encompassing approximately 40 per cent of continental United States.

Numerous species of wild rodents have been implicated in the epizootiology of plague since the first suggestive evidence of an epizootic in ground squirrels, *Citellus beecheyi*, was found in Contra Costa County, California in 1903–1905 (Meyer, 1942). When plague was conclusively demonstrated in ground squirrels by McCoy (1908) and Wherry (1908), the attention of investigators was focused upon the larger colonial rodents. Thus Eskey and Haas (1940), in their classical study of plague in the western part of the country, indicated that the primary reservoirs of plague were ground squirrels, *Citellus* spp.; wood rats, *Neotoma* spp.; and prairie dogs, *Cynomys* spp. Although these and other authors mentioned occasional findings of plague in native mice, such as *Peromyscus* spp., they were of the opinion that these were unimportant accidental infections acquired by contact with ground squirrels or other important reservoirs.

Colonial rodents best served the purpose of the early plague surveys which were conducted primarily to demonstrate the geographic extent of the disease. The adoption in 1936 of routine inoculation of pooled fleas in addition to tissue inoculations by Eskey and Haas (1940) increased the efficiency of surveys to delineate the rodent and flea reservoirs of plague. Consequently, tissue and flea pools from ground squirrels and prairie dogs constitute the major source of plague positive records thus far accumulated on wild rodent plague in this country.

In more recent years there has been a growing conviction among plague workers that rodents other than the ground squirrel and the large conspicuous species may be more usual reservoirs of *P. pestis*. The colonial rodents are perhaps, like man, only occasionally included in plague outbreaks which are initiated by epizootics among native field mice (Meyer, 1955). In California, since 1908, investigations have shown that plague in wild rodents was enzootic in three-fourths of the state, and by 1952 the following 10 genera of native wild rodents and 2 genera of lagomorphs and/or their fleas were implicated with plague: *Citellus*, *Neotoma*, *Peromyscus*, *Eutamias*, *Tamiasciurus*, *Glaucomys*, *Marmota*, *Thomomys*, *Microtus*, *Reithrodontomys*, *Lepus*, and *Sylvilagus* (Jones and Street, 1956). A perusal of the California records shows that plague-positive fleas have been taken from the small native field mice on numerous occasions. For instance, during 1943, in Monterey County, meadow mice (*Microtus californicus*), white-footed mice (*Peromyscus* spp.), and their fleas, were discovered to be infected with plague in an area where ground squirrels were thought to have been virtually eradicated by intensive control operations (Jones and Street, 1956).

Epizootiological studies in various parts of the world have indicated that the mammalian plague reservoirs of primary importance are not necessarily the highly susceptible conspicuous species. Some native mice which are relatively

plague-resistant may constitute the more usual reservoirs for the persistence of plague as has been found in Africa (Heisch *et al.*, 1953; Davis, D. H. S., 1953). Preliminary findings in California (Quan and Kartman, 1956) and in New Mexico (Holdenried and Quan, 1956) suggest that certain strains of native field mice, *Microtus* spp. and *Dipodomys* spp., are resistant to experimental plague infection.

It is important to note that some of these small rodents are generally more closely associated with domestic rats, *Rattus* spp., in the immediate environs of human habitations, than are the ground squirrels and other colonial species. Thus the potential danger is obvious and, in the words of Meyer (1955), "The increase in the population of California has given rise to two situations that must be taken into account. The building of suburbs ultimately makes a habitat unsuitable for maintaining appreciable wild rodent populations, but there is at least an initial period of joint tenancy by people and wild and commensal rodents—a condition theoretically ideal for the propagation of plague. The wilderness recreational areas of California have been crowded with more people every year. This too has increased the opportunity for close contact between man and reservoir animals and ectoparasites."

It is known that native field mice and domestic rats coexist and live in newly suburbanized areas, around refuse disposal sites, on ranches, in recreational areas, and even in city vacant lots. If plague-infective fleas from field mice transfer to rats, causing plague, then infective rat fleas are introduced into the human environment. The dangers inherent in the close association of wild rodents and domestic rats was recognized over 40 years ago by McCoy (1910). Eskey (1938) found the wild rodent flea, *Malaraeus telchinum*, on Norway rats in San Francisco. Prince (1943), from an analysis of data based on 5,785 fleas from 4,188 domestic rats caught in 13 western states, showed that these rats harbored 15 different species of wild rodent fleas. Meyer and Holdenried (1949) established the existence of plague in rats, *Rattus r. rattus* and *R. norvegicus*, and in ground squirrels, *Citellus beecheyi*, on a ranch in Ventura County, California. Rat fleas were not found on the squirrels, but one quarter of the fleas taken from the rats were squirrel fleas, *Diamanus montanus* and *Hoplopsyllus anomalus*. Thus these authors concluded that plague was transferred to the rats by these wild rodent ectoparasites.

While the transfer of plague from wild rodents to domestic rats constitutes the most serious potential for human outbreaks, infection of humans directly from wild rodent sources is a secondary threat. Pollitzer (1954) lists 91 plague cases and 57 deaths in 7 western states during the period 1908–1951 as having been contracted from wild rodents or their fleas. In June, 1956 a fatal human case of plague occurred in Ventura County, California which was apparently contracted from ground squirrel fleas.

Although the above historical account has merely presented a few of the more important facts concerning plague in the western region, it is sufficient to indicate that plague studies in this country have only scratched the surface of the formidable epizootiological complex represented by the phrase "wild rodent

plague." There is a definite need for intensive ecological studies to elucidate the host-ectoparasite-disease relationships in plague foci, especially those found near human populations. Thus the hazard of plague to the public health at present cannot be accurately evaluated and only ill-defined recommendations can be made for safeguarding human beings against the enzootic plague reservoir. It is generally agreed that, while it is impracticable to attempt the control of wild rodent plague on a wide scale, natural plague foci should not be permitted to exist where they threaten human populations. The potentially dangerous nature of natural focal localizations or "nidi" of diseases transmissible to man have been described in detail by Pavlovsky (1955). Thus it is now somewhat vaguely suggested that plague-free zones should be maintained by elimination of foci around populated areas or places temporarily inhabited by man. This concept is confronted at the outset by the paucity of swift techniques for determining the presence or absence of plague in a given area. Furthermore, the currently meager knowledge of the ecology of plague precludes a sound judgment of such basic considerations as the weakest link in the infection chain, the seasons of prevalence and quiescence, practical effective dimensions of protective zones, and the types of habitats, reservoirs, and wild-domestic rodent contacts which may locally harbor plague and present a threat to humans.

Pioneering work on plague ecology in California has been done by a group of scientists formerly at the University of California, Hooper Foundation (1939–1946), under the direction of K. F. Meyer. These studies did much to show the direction in which modern concepts of plague ecology must travel. More recently, the U. S. Public Health Service established a field station at Santa Fe, New Mexico (Holdenried, 1951) where studies of plague ecology in small native rodents were carried out during a three year period.

The present studies were initiated in March, 1954 to study intensively a plague focus in the San Francisco Bay area where native species of field mice were closely associated with domestic rats in proximity to human habitations. The particular plague-enzootic area was found during a preliminary survey begun one year earlier to investigate the occurrence of plague in populations of wild rodents closely associated with domestic rats in the San Francisco Bay region, and to study their flea-host relationships (Miles *et al.*, 1957). The major objective was to investigate the ecology of plague as reflected by: 1—the physical limits of the focus; 2—the flea and rodent species involved; 3—the natural plague infection rates of different flea species; 4—the plague susceptibility of the rodents; 5—seasonal variations in plague prevalence; and 6—effects upon the rodent population.

THE STUDY AREA

The studies were conducted adjacent to the Central Hog Farm on the southwestern slopes of San Bruno Mountain in northeast San Mateo County (Fig. 1). San Bruno Mountain occupies a small rugged area extending northwestward from South San Francisco to Daly City, a distance of about 5 miles. The highest point in this area is 1,315 feet (Davis, 1955). The city of Colma, which is 2½

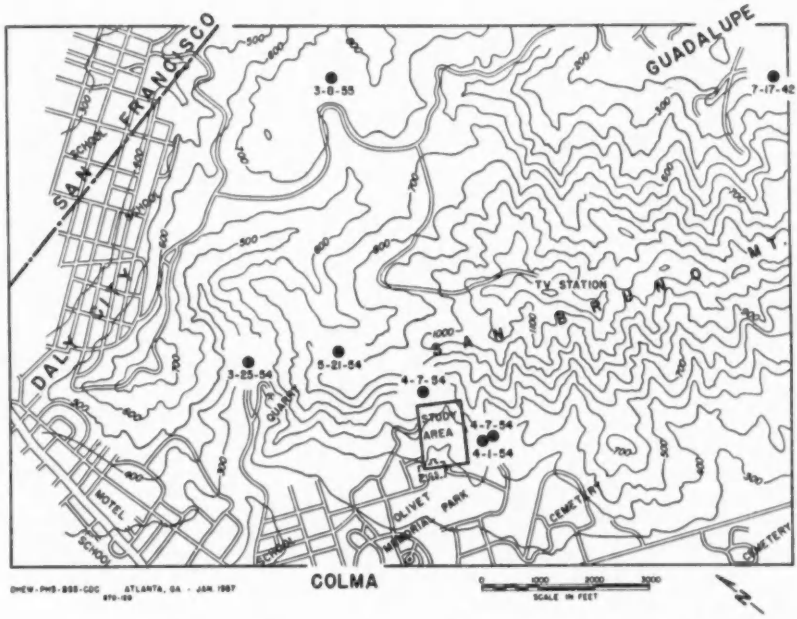

FIG. 1. Map showing location of the study area in San Bruno Mountain in relation to adjacent communities and location of known enzootic plague foci (black circles) with dates of determination.

miles south of the San Francisco city limits, lies adjacent to the south slope of the mountain and the Guadalupe Valley occurs at its foot on the northeastern side. Actually, the San Bruno Mountain is the northern block of the Santa Clara Mountains, separated from the southern block, Montara Mountain, by the San Bruno fault lying at the base of the steep southwestern front of San Bruno Mountain (Hinds, 1952). Geologically, the area is classified in the upper Jurassic age characterized by dark marine sandstone, dark shale, basalt and greenstone, glaucophane schist, limestone, and chert (Davis, 1955).

Climate

A 22-year record for San Mateo County shows a maximum temperature of 104° F. and a minimum of 23° F. with averages in January of 50° F., and in July of 68° F. Killing frosts occur in late December and end early in February. A 21-year record shows an annual average precipitation of 22 inches with averages for January of 6 inches; May, 0.63 inches; July, 0.01 inches; September, 0.32 inches; and November, 2 inches. The growing season is about 319 days (Yearbook of Agriculture, 1941). Hygrothermographic records from the study area show the following data (Table 1).

TABLE 1

	Temperature in °F			Relative humidity
	Maximum	Minimum	Average	Percent
April	77	46	59.1	82.6
May	68	43	55.2	77.5
June	74	44	57.8	73.4
July	82	50	62.7	68.7
August	70	50	57.9	75.1
September	86	39	55.9	67.1
October	72	48	60.4	70.6

Vegetation Types

The types of vegetation and the ecologic association of floral and faunal species have been reviewed by Hooper (1944) in an extensive discussion of rodent speciation in the San Francisco Bay region. In brief, the major vegetation types of the San Bruno Mountain area may be summarized as follows.

1—Grassland: open shrub grass with widely scattered coyote brush (*Baccharis pilularis*) or brush lupine grass distributed widely; heavily grazed annual grasses broadly covering the eastern half of the south slope with brome grasses (*Bromus* spp.), wild oats (*Avena barbata*); restricted patches of ungrazed or little grazed annual or perennial grasses, occasionally a mixture with poison hemlock (*Conium maculatum*), cow parsnip (*Heracleum lanatum*) or various herbs forming a dense cover; bur clover (*Medicago hispida*) is widely scattered.

2—Soft chaparral: this is a shrub formation of the transition life zone, mainly brush (*Baccharis pilularis*), monkey flower (*Diplacus aurantiacus*), and coffee berry (*Rhamnus californicus*) on slopes to south and west.

3—Coastal brush: dense low tangle of coyote brush, blackberry (*Rubus vitifolius*), lizard tail (*Eriophyllum staechadifolium*) mixed with herbs on much of the west and southwest slopes mainly in canyons.

4—Riparian: thickets that form narrow growth along creeks with plants restricted to moist soils, as willow (*Salix* sp.).

The specific ecologic associations of vegetation and rodent species in the San Bruno Mountain area have been discussed by Murray (1957) who conducted a survey of rodent-ectoparasite-disease relations in the area during a portion of the period in which the present study was under way. An extensive account of the flora is given by Howell (1949).

Rodent Species

The species of rodents and lagomorphs encountered in the study area were as follows: the grey rat (*Rattus norvegicus*), the California vole (*Microtus californicus*), the western harvest mouse (*Reithrodontomys megalotis*), the deer mouse (*Peromyscus maniculatus*), the house mouse (*Mus musculus*), and the California brush rabbit (*Sylvilagus bachmani*) together with several other small mammals of no particular concern here.

Flea Species

The species of fleas concerned in this study were as follows: *Malaraeus telchinum, Catallagia wymani, Hystrichopsylla linsdalei, Nosopsyllus fasciatus, Atyphloceras multidentatus,* and *Opisodasys keeni nesiotus.*

METHODS AND MATERIALS

Figure 2 is a diagram of the plague-enzootic area selected for this study beginning March, 1954. A 200 x 600 foot plot was selected along a small waterway on the uncultivated lower slopes of the mountain at an elevation of from 375 to 500 feet. The watercourse was dry except during the winter and spring rainy seasons. The wild type habitat was continuous across the study area and along the watercourse and gave way to a willow grove adjacent to the premises of the hog farm. There was a small amount of tillage, principally for commercial flower growing, which reached the area mainly at its lower end. Figure 3 presents views of typical situations in the area.

Trap sites were established in a grid pattern at 50-foot intervals throughout the study plot (lines A–E, Fig. 2). A single line (R) was continued down stream to the hog feeding pens; operation of this line was intermittent because of periodic rat control activities at the hog farm. Trap lines P and S, along each side of a secondary watercourse, were operated for six weeks only at the beginning of the study.

From May through October, 1954, additional traps were operated in a portion of the principal study plot as a temporary check on the adequacy of trap coverage. Traps were placed at 25-foot intervals midway between trap sites 1 through 4 in lines B, C, and D, and likewise between trap lines B to E. Thus a grid was established over an area of about 125 × 150 feet.

Cage traps 4″ × 4″ × 15″ (Young's Animal Trap Co., Sunnyvale, California) were used throughout the studies. A retreat box was added to the traps in the early part of the studies to provide shelter for captured small mammals; the Young trap, as modified, is described elsewhere (Kinney *et al.*, 1957).

The traps were set usually for 2 or 3 consecutive nights each week and were baited with dry rolled oats. On visiting the traps, the necessary equipment was carried in the field for processing each mammal at its capture site. Mammals captured were lightly etherized in a glass jar, then transferred to a white enameled pan for removal of fleas by use of a typewriter brush. The cotton bedding was then removed from the retreat box and thoroughly examined for fleas. The fleas from each mammal and from its bedding were placed in 2 per cent aqueous NaCl in separate 1-dram vials by use of a small camel's hair brush. The cotton bedding, if dry and reasonably clean, was returned to the retreat box, but was discarded if soiled or wet.

The mammals were individually marked by a system of toe clipping previously described by Ecke (1955). On each capture, the rodents were measured (combined length of head, body, and tail) and evidence of reproductive activity was noted as follows; females were palpated, and the size of embryos, if evident, was recorded as one of 3 coded categories, *i.e.*, small, medium or large, to desig-

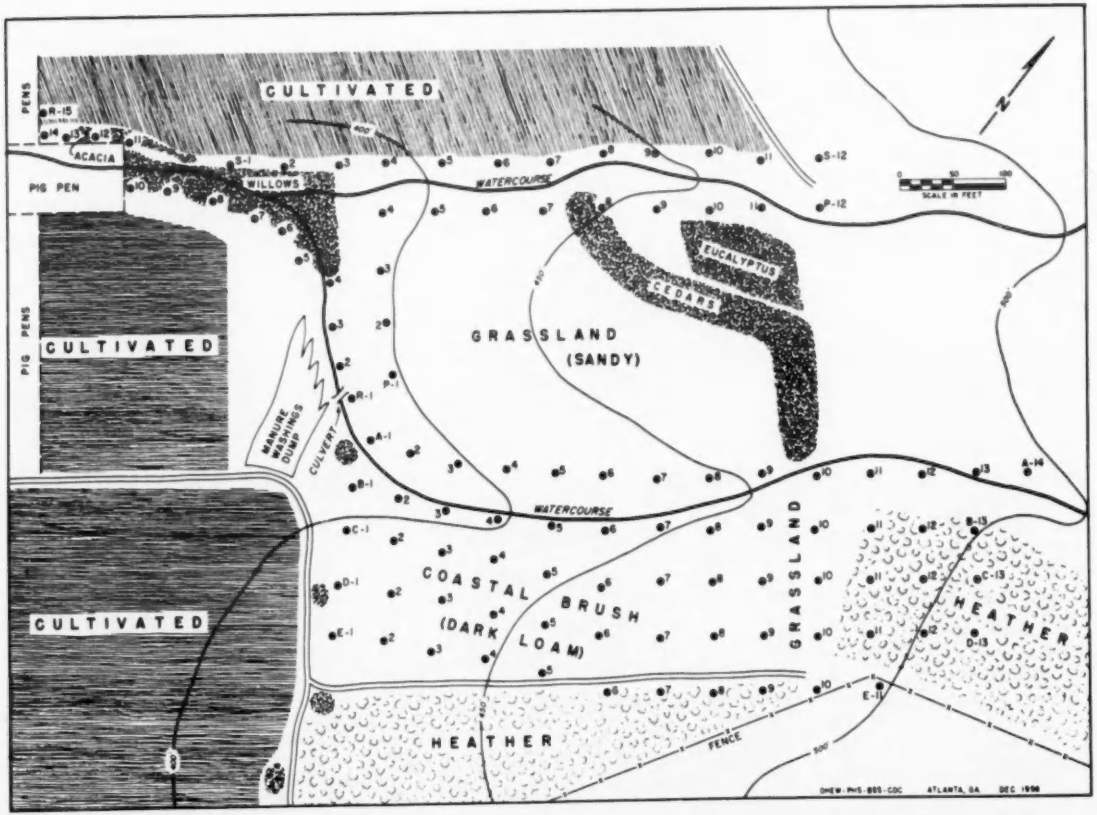

FIG. 2. Diagram of the study area showing trap lines, surrounding vegetation, and location of hog pens.

FIG. 3. Typical situations in the study area: A—general view northeast from the hog farm showing hog pen in foreground and a portion of San Bruno mountain in background; B—close up of same view northeast showing field men checking trap sites along water course; C—looking northeast along water course shown descending from mountain, trap site in foreground; D—field man checking trap site in heavy cover; E—view southwest showing heavy grass cover in foreground and hog farm buildings in background; F—southwest view showing willow grove habitat of Norway rats adjacent to hog farm structures.

nate early, mid-term or late-term pregnancy respectively; the condition of mammae was similarly recorded as evidence of reproductive activity indicated by (1) mammae small and concealed by pelage, (2) exposed and prominent, thus suggesting recent lactation, and (3) currently lactating. Evidence of current mating habits of males was noted by the scrotal or abdominal position of testes.

Mammals which appeared normal were released at the sites of their capture. The tissues of rodents found dead or moribund were examined in the laboratory for the presence of *P. pestis*, except occasional ones obviously overexposed to heat in traps near mid-day.

The inoculation of laboratory animals with flea pools does not provide definitive data on natural plague infection rates in fleas. Accordingly, a practical method was devised for detecting *P. pestis* infection in individual fleas by agar plate culture. All fleas collected during six consecutive weeks, beginning the 2nd week of study, and during a similar period of high plague prevalence in February and March, 1955, were individually cultured. In the interim period, this method was used only intermittently on the weekly collections of fleas, since its continuous use did not seem justified when less than one per cent of the fleas were indicated as plague-positive. This method was used alternately with inoculations of flea pools into animals.

All wild rodents taken in the first eight weeks, and all *Rattus* captures throughout the study, were examined for fleas; fleas were collected from only one or 2 days' native rodent captures in each week after May 1, 1954, however, and those taken in the extra traps were not brushed.

RESULTS

Collections prior to studies by live trapping. Plague was first detected in the study area in July, 1953 during studies of fleas from snap-trapped mammals (Miles *et al.*, 1957). An account of the trappings in this particular area is here summarized, however, as background information on the occurrence of rodents, fleas and plague during the year preceding the live-trapping studies. It should be noted that other plague foci were found in the San Bruno Mountain region both prior to, and after 1953 (Fig. 1) by the California State Bureau of Vector Control and by this station.

The area was trapped with Victor rat traps and Museum Special traps (50 each) in 3 trapping periods at about 3-month intervals. Approximately the same area was covered in each trapping; that is, the trap lines extended from the pig pens and along the small stream for a distance of about 500 feet (R 10 to B 5, Fig. 2). The fleas collected were pooled by host species and inoculated into laboratory animals for isolation of *P. pestis*. The rodents and fleas taken in each of 4 trapping periods and the results of flea pool inoculations are shown in Table 2.

The 2 plague positive flea pools consisted of: I. 36 *Malaraeus telchinum*, 8 *Catallagia wymani*, 6 *Hystrichopsylla linsdalei*, 7 *Nosopsyllus fasciatus* and 1 *Atyphloceras multidentatus* collected from 14 *M. californicus*; II. 6 *Opisodasys keeni nesiotus* and 1 *M. telchinum* collected from 3 *P. maniculatus*.

TABLE 2

Rodent species	Number of rodents taken on dates shown			
	3/31–4/1/53	7/29–7/31/53	10/7–10/9/53	1/13–1/15/54
Rattus norvegicus	26	5	0	0
Microtus californicus	13	17	30	82
Reithrodontomys megalotis	5	9	33	21
Peromyscus maniculatus	3	3	2	14
No. fleas on all rodents	75	99	177	354
Plague positive pools	0	2	0	0

TABLE 3

Flea species	Number rodents from indicated trap sites		
	B 1 to B 3 C 1 to C 3	B 6 and B 7	B 8, B 9, and C 8
	5 *M. californicus* 1 *P. maniculatus*	2 *M. californicus* 1 *R. megalotis*	3 *P. maniculatus*
M. telchinum	12	23	6
H. linsdalei	1	1	0
A. multidentatus	2	1	1
C. wymani	0	0	1
O. k. nesiotus	0	0	3
Total fleas	15	25	11

These infections could not be accurately located within the area since the pooled fleas were widely distributed in the trap lines. Plague was again found, however, in 2 pools of fleas collected from *Microtus* nests taken near the locations later designated as live-trap sites R 2 and A 14 (Fig. 2). The 2 flea pools consisted, respectively, of 19 *M. telchinum*, 1 *A. multidentatus* and 5 *H. linsdalei* collected August 13, 1953; and 22 *H. linsdalei* collected February 25, 1954.

Preliminary studies by pooling of fleas from live trapped rodents. Although plague was not found in the fleas collected from rodents snap-trapped in October, 1953 and in January, 1954, it appeared evident that plague was enzootic in the area. Studies were therefore begun on March 10, 1954, with live traps set at the locations B 1 to B 10 and C 1 to C 10 (Fig. 2). The fleas were pooled by sites of collection along the trap lines. Plague was isolated from each of 3 flea pools representing trap site groups as shown in Table 3.

One *M. telchinum* from one *R. megalotis* trapped at B 10 was negative for plague. Of 4 fleas collected from one *M. californicus* at B 4, one *H. linsdalei* was negative, while a pool of 3 *M. telchinum* was positive for plague.

The presence of plague infection was thus shown in fleas collected from at least 4 locations along trap line B. Fleas collected in the next trapping, March

18 and 19, were separated into smaller pools, both by species and by trap sites, in order to secure more specific data. Additional trap lines also were established as follows: A 1 to 14 (March 18), D 1 to 13 and E 1 to 4 (March 19). In a total of 34 pooled inoculations of 100 fleas collected from 21 hosts, plague was isolated from 5 pools as follows:

10 *M. telchinum* from 2 *M. californicus* at A 6
1 *O. k. nesiotus* from 1 *M. californicus* at A 6
7 *M. telchinum* from 2 *M. californicus* at B 4
2 *H. linsdalei* from 1 *M. californicus* at B 4
6 *M. telchinum* from 1 *M. californicus* at C 9

Additional locations in the area were thus shown to be plague-infected. Fleas in the 29 plague-negative pools were collected from 5 *M. californicus*, 5 *P. maniculatus*, 5 *R. megalotis* and 1 *Sylvilagus bachmani*, widely distributed in the trap lines.

All rodents observed in the area appeared normal except that occasional ones were found dead in traps, apparently from exposure to the elements. Tissue inoculations from 3 *M. californicus*, dead in traps on March 19, were plague-negative.

It was evident from these preliminary observations that *P. pestis* was unusually prevalent in the area. An opportunity thus was found for detailed studies of wild rodent plague in close proximity to human beings.

Observations on the involvement of the contiguous rat population, a factor of prime epidemiological concern, were minimal because of the prompt action taken by local health authorities to control *Rattus*. That plague actually involved the rats associated with native wild rodents was shown by the isolation of *P. pestis* from the tissues of a *Rattus norvegicus* found dead in trap R3 on April 12, 1954.

During the ecological studies that followed the above preliminary work, the cooperation by local health authorities in limiting rodent control activities to the immediate environs of the hog farm, permitted a continuation of observations in the undisturbed wildtype habitat. However, tillage of the study area for commercial flower cultivation during January, 1955, forced a termination to the major studies. The papers that follow in this series present the principal findings concerning the rodent populations, the flea species, the plague infection rates in the fleas, and the susceptibility of the rodents to plague.

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ON THE SIZE AND SHAPE OF THE EGGS OF THE GEOGRAPHIC STRAINS OF *SCHISTOSOMA JAPONICUM*[1]

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The strain complex of *Schistosoma japonicum* has become an interesting subject of investigation in the past few years. Differences in the infectivity of the various geographic strains of this parasite have been demonstrated with its intermediate hosts *Oncomelania* spp. (Hunter *et al.*, 1952; DeWitt, 1954). It has been proved that the Formosan strain of *S. japonicum* cannot develop to maturity in man (Hsü and Hsü, 1956), and that the Formosan monkey, *Macaca cyclopis*, is usually refractory to it (Hsü and Hsü, 1956a). Strain variations in the number and arrangement of testes, and hermaphroditism in the male worms have been found (Hsü and Hsü, 1957). The size and shape of the eggs of a parasite are usually regarded as important diagnostic characteristics. It has been demonstrated that it is possible to differentiate between some of the closely related schistosomes of Africa in this way (Alves, 1949; Amberson and Schwarz, 1953; Schwetz, 1953). The present investigation was undertaken to determine whether similar differences exist between the *S. japonicum* strains.

MATERIALS AND METHODS

Four geographic strains of *S. japonicum* were used: the Chinese, Formosan, Japanese, and Philippine. The cercariae of the Chinese strain were obtained from *Oncomelania hupensis* originating in Kashing, Chekiang, China; the Formosan from *O. formosana* obtained from Changhua, Taiwan; the Japanese from *O. nosophora* obtained from Kurume, Kyushu, Japan; and the Philippine from *O. quadrasi*, obtained from Palo, Leyte, the Philippines. Infections in the mammals used in the experiments were the result of laboratory exposure to cercariae. The egg measurements were started on the fourth day after the stools of the animals were found positive for mature eggs. When possible, all of the measurements were made on eggs produced during a single day. When the number was insufficient, the measurements were made on the following day(s). The eggs were concentrated by sedimentation in normal saline. Measurements were also made in saline. Only mature and properly oriented eggs were measured. A slight tap on the cover glass with a needle often was sufficient to right tilted eggs. The width/length $\times$ 100 was used as an index of egg size. Unless otherwise noted, the 5 per cent level of significance was used in evaluating the results of the statistical tests.

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RESULTS

Interstrain variations

Three of each of the following—dog, cat, hamster and albino mouse—were infected with each of the three strains of the parasite—the Chinese, Formosan and Japanese. Because of a shortage of cercariae, only three mice were infected with the Philippine strain. The data obtained from the measurements of 300 eggs from each animal are given in Table 1.

The analysis of variance method was used in the comparisons. The first F-tests compared the variation of the eggs *among* the three host-individuals of one host-species infected with one strain of the parasite, to that *within* each of the three host-individuals. Each value represented one egg in the test. The results of these tests showed that in nearly all cases there were significant differences in length, width and index of eggs between the host-individuals.

Since there were these significant differences in the eggs from different individ-

TABLE 1

The mean and standard error (= standard deviation of the mean) of measurements of 300 eggs of Formosan, Chinese, Japanese, and Philippine strains of S. japonicum *from each host animal (measurements in μ)*

S. jap. strain	Measure	Mean and standard error of 300 eggs											
		Dog			Cat			Hamster			Mouse		
		1	2	3	1	2	3	1	2	3	1	2	3
Formosan	Length	91 ±.3	89 ±.3	89 ±.3	88 ±.3	84 ±.3	88 ±.3	87 ±.2	88 ±.2	88 ±.3	86 ±.2	84 ±.2	82 ±.2
	Width	68 ±.2	66 ±.2	64 ±.2	70 ±.3	66 ±.2	64 ±.2	60 ±.2	61 ±.2	61 ±.2	61 ±.2	59 ±.2	59 ±.2
	Index	73 ±.3	74 ±.3	72 ±.3	80 ±.3	79 ±.3	73 ±.3	71 ±.3	69 ±.3	70 ±.3	71 ±.3	70 ±.3	72 ±.2
Chinese	Length	87 ±.2	89 ±.4	86 ±.3	87 ±.2	86 ±.3	89 ±.3	90 ±.3	88 ±.3	90 ±.2	87 ±.4	86 ±.3	87 ±.3
	Width	60 ±.2	59 ±.3	59 ±.2	58 ±.2	60 ±.2	60 ±.2	59 ±.2	59 ±.2	58 ±.2	58 ±.2	57 ±.2	58 ±.2
	Index	69 ±.3	68 ±.4	68 ±.3	67 ±.2	70 ±.3	67 ±.2	66 ±.3	67 ±.3	65 ±.3	66 ±.4	67 ±.3	67 ±.3
Japanese	Length	81 ±.2	83 ±.2	80 ±.3	78 ±.3	77 ±.2	77 ±.2	80 ±.3	79 ±.3	83 ±.3	82 ±.3	81 ±.2	80 ±.2
	Width	64 ±.2	65 ±.2	62 ±.2	61 ±.2	60 ±.2	59 ±.2	60 ±.2	60 ±.2	62 ±.2	62 ±.2	61 ±.2	59 ±.2
	Index	79 ±.3	79 ±.2	77 ±.3	78 ±.3	78 ±.3	76 ±.3	75 ±.3	76 ±.3	75 ±.3	75 ±.3	76 ±.3	75 ±.3
Philippine	Length	No infected animals									83 ±.3	85 ±.3	83 ±.3
	Width										57 ±.2	61 ±.2	58 ±.2
	Index										69 ±.3	73 ±.3	70 ±.3

uals of the same species infected with the same strain of the parasite, the mean of the measurements of the three hundred eggs from one host-individual was used as one value in the F-tests to compare the variation of the size and shape of the eggs *among* different strains of the parasite to that *within* the strains.

The F-tests on the interstrain variations of the eggs have been done separately for the length, width, and index. As the data concerning the eggs of the Philippine strain were obtained only from the mouse, the part of the analysis concerned with the eggs from all four host-species has been confined to the other three strains.

1. *Length of the egg* (Table 1 and Fig. 1). In all four species of the hosts, the average length of eggs of the Formosan and Chinese strains was greater than that of the Japanese. The F-tests showed that in dogs, cats and hamsters this difference was significant, but not in mice. As the eggs of the Japanese strain from the cat were especially short, the differences between the length of eggs of the Japanese strain and that of the Formosan and Chinese strains were especially marked. Between the Formosan and Chinese strains, the eggs of the Formosan strain from the dogs were significantly longer than those from the Chinese strain in this host. In hamsters, cats and mice the eggs of the former were shorter, but the differences were not significant. The average length of the eggs of the Philippine strain from the mice was less than that of the Chinese and Formosan, but greater than that of the Japanese. The difference was significant between the Chinese and Philippine and between the Philippine and Japanese, but not between the Formosan and Philippine.

2. *Width of the egg* (Table 1 and Fig. 1). In all four species of the hosts, the average width of eggs of the Formosan and Japanese strains was greater than that of the Chinese. The F-tests indicated that in hamsters and cats the difference between the Formosan and Chinese strains were significant, but the difference between the Japanese strain and the latter was not. In mice, the average width of the Japanese strain was significantly greater than that of the Chinese, but that of the Formosan was not. In dogs, cats and hamsters the width of the eggs of the Formosan strain was greater than that of the Japanese, but in mice it was less. None of these differences were significant. The average width of the eggs of the Philippine strain in mice was less than that of the Japanese and Formosan, but greater than that of the Chinese strain. None of these differences were significant.

3. *Index of the egg* (Table 1 and Fig. 1). In all the four host-species the indices of eggs of the four strains fell into the following order: Japanese > Formosan > Philippine > Chinese. The F-tests showed that in dogs, cats and hamsters, the differences of the indices of eggs between different strains were significant. In mice this difference was not significant between Formosan and Philippine strains, although it was between other strains.

The above data indicate that the eggs of the Japanese strain are broad and short, giving a high index. The eggs of the Chinese strain are narrow and long, giving the lowest index. The other two strains lie between these two extremes, in the order given above.

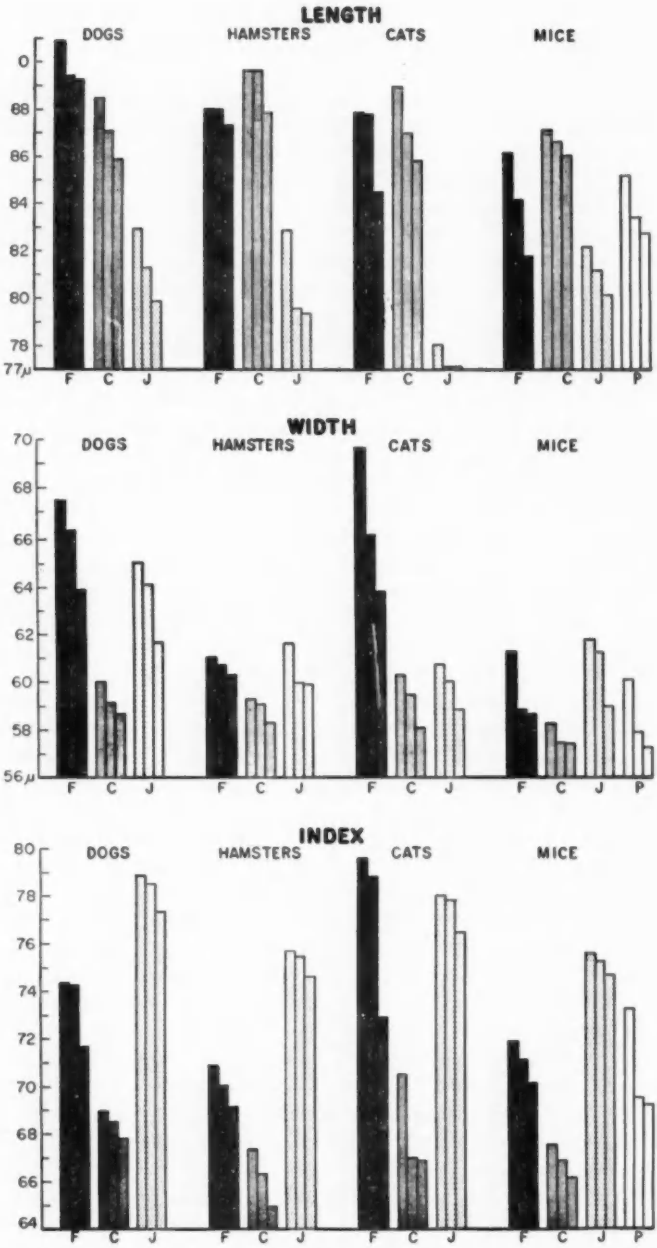

FIG. 1. Histogram showing the variations of the means of measurements of 300 eggs of various *S. japonicum* strains from different hosts. Each subdivided column represents the mean of the measurements of 300 eggs from one host-individual. F means the Formosan strain; C the Chinese; J the Japanese; and P the Philippine.

Variations between host-species

That there is a variation of the size and shape of the eggs of *S. japonicum* from different host-species has been noted above. In a detailed study of these variations the mean of the measurements of 300 eggs from each host-individual was used. The eggs of the Chinese, Formosan, and Japanese strains from the dog, cat, hamster, and mouse were compared by the methods described above.

1. *Length of the eggs* (Table 1). The average lengths of the eggs of the Chinese strain from the different species of hosts fell into the following order: hamster > cat > dog > mouse. The difference was significant only between hamsters and mice. In the Formosan strain, the order was: dog > hamster > cat > mouse. The difference was significant only between the dogs and hamsters, and between dogs and mice. In the Japanese strain the order was: dog > hamster > mouse > cat. The eggs from cats were very short and the differences between them and the eggs from dogs and mice were significant. None of the other differences were significant with this strain.

2. *Width of the eggs* (Table 1). The average width of the eggs of the Chinese and Formosan strains from the different host-species followed the same pattern: cat > dog > hamster > mouse. In the Japanese it was quite different: dog > mouse > hamster > cat. The average width of the eggs of the Chinese strain from cats was greater than that from dogs, hamsters, and mice. The difference was significant only between cats and mice. The eggs from mice differed significantly from those from dogs and hamsters. The average width of the eggs of the Formosan strain from cats was greater than that from dogs, hamsters and mice. The difference was significant only between cats and hamsters and between cats and mice. The average width of the eggs from dogs was significantly different from that from hamsters and mice. The average width of the eggs of the Japanese strain from dogs differed significantly from that from hamsters, cats and mice. None of the other differences were significant with this strain.

3. *Index of the egg* (Table 1). The average indices of the eggs of the three strains from the four species of hosts fell into the following order: cat > dog > mouse > hamster. In the Formosan and Japanese strains the difference was significant only between cats and mice, cats and hamsters, dogs and mice, and dogs and hamsters. In the Chinese strain, the difference of the index of the eggs was significant only between dogs and mice, and between dogs and hamsters.

In general the eggs from cats and dogs have a higher index than that from mice and hamsters. This results from the fact that the width of the eggs from the former two species was greater than those from the latter two. Although the width of the eggs from cats infected with the Japanese strain was not greater than that from mice and hamsters, the eggs of this strain from the cat were especially short, so that the index of the eggs was still higher than that from mice and hamsters.

Daily variations

Since it was possible that daily variations in the size and shape of the eggs might affect the data, daily measurements were made of 50 mature eggs for 30 consecutive days from each of three cats. One was infected with the Chinese, one

with the Formosan, and the other with the Japanese strain. The analysis described above was used on the data obtained. Measurements of individual eggs were used as the values in comparing the variations of the eggs *among* 30 days to that *within* each day. In general there were significant daily differences between the eggs of the three strains, except for the index of the Japanese strain. The daily variation of the means were irregular, showing no definite trend of increase or decrease (Table 2). The mean of the 30 means of the 50 eggs was calculated for each of the three strains (Table 2). A comparison of the means of the means

TABLE 2

The daily mean of measurements of 50 eggs of S. japonicum *from each of the 3 cats infected with the Formosan, Chinese, and Japanese strains respectively. The measurements were done for 30 consecutive days (measurements in μ). F: Formosan strain; C: Chinese; J: Japanese*

Days (consecutive)	Mean of 50 eggs								
	Length			Width			Index		
	F	C	J	F	C	J	F	C	J
1	90	82	80	65	59	61	72	71	76
2	93	85	78	68	61	60	74	72	76
3	93	84	79	69	60	61	74	72	78
4	94	85	79	71	59	62	75	70	79
5	95	86	79	70	60	62	75	70	79
6	94	87	79	69	60	62	74	69	78
7	91	86	78	69	58	60	76	68	77
8	89	85	78	65	60	62	73	71	79
9	88	87	79	65	61	61	75	70	78
10	89	85	78	69	60	61	73	71	78
11	88	82	81	86	59	62	75	72	76
12	89	86	80	66	59	63	75	69	79
13	90	87	78	69	59	61	73	69	78
14	91	85	78	70	61	62	78	72	79
15	96	85	79	74	60	62	77	71	78
16	92	86	79	71	62	62	77	73	79
17	91	86	80	70	61	62	77	71	78
18	92	90	82	73	65	63	80	72	77
19	90	87	79	70	59	62	79	68	79
20	94	88	78	72	60	62	76	68	80
21	94	88	80	71	61	62	76	70	77
22	95	87	79	76	60	61	80	69	77
23	91	87	79	71	60	61	78	69	77
24	94	89	79	71	62	63	76	70	80
25	95	84	79	69	60	62	74	71	79
26	95	87	80	73	60	63	77	69	79
27	94	88	78	73	60	62	77	69	79
28	93	88	80	69	61	63	75	70	78
29	90	87	79	69	59	62	76	68	78
30	87	88	79	66	63	62	76	72	78
$\bar{x} \pm \sigma_{\bar{x}}$	92 ± .5	86 ± .3	79 ± .2	70 ± .5	60 ± .3	62 ± .1	76 ± .4	70 ± .3	78 ± .2

of the three strains showed that the size and shape of the eggs fell into the following order: for length, Formosan > Chinese > Japanese; for width, Formosan > Japanese > Chinese; and for index, Japanese > Formosan > Chinese. The t-test showed that the differences between the means of the means of the length, width and index of the three strains were significant. A comparison of these results with those obtained from the three means from three cats infected with

TABLE 3

The mean of measurements of 50 eggs of S. japonicum *from each of the 97 mice: 30 mice were infected with the Chinese strain, 30 with the Formosan, 30 with the Japanese, and 7 with the Philippine. C: Chinese strain; F: Formosan; J: Japanese; and P: Philippine (measurements in μ)*

No. of mice	Mean of 50 eggs											
	Length				Width				Index			
	C	F	J	P	C	F	J	P	C	F	J	P
1	87	82	81	83	57	59	60	56	65	72	74	68
2	86	85	81	85	58	60	61	62	67	71	75	73
3	87	85	83	87	58	61	63	61	67	72	75	70
4	88	84	79	83	57	61	60	57	65	72	77	69
5	84	83	79	84	56	63	61	58	66	76	78	68
6	84	84	79	82	58	62	61	57	69	74	77	70
7	87	82	80	82	57	60	61	57	66	74	76	70
8	84	83	83	—	58	61	63	—	68	74	76	—
9	86	85	83	—	56	62	63	—	65	73	76	—
10	85	82	82	—	57	59	63	—	67	72	78	—
11	86	81	82	—	56	61	63	—	65	75	77	—
12	86	82	83	—	56	59	63	—	66	72	76	—
13	87	85	84	—	57	64	63	—	65	75	75	—
14	87	86	83	—	56	62	62	—	65	73	75	—
15	86	82	83	—	56	61	64	—	65	75	76	—
16	85	82	82	—	57	59	62	—	67	72	76	—
17	87	81	84	—	58	59	64	—	67	73	75	—
18	86	84	83	—	56	62	64	—	65	73	77	—
19	87	82	84	—	56	60	63	—	65	73	75	—
20	87	82	83	—	57	60	63	—	66	74	77	—
21	89	84	83	—	58	61	63	—	64	72	76	—
22	85	82	85	—	59	61	63	—	69	74	74	—
23	88	85	79	—	58	61	61	—	65	72	78	—
24	84	83	86	—	57	63	64	—	68	76	75	—
25	88	86	85	—	57	62	64	—	65	72	75	—
26	87	84	83	—	57	62	64	—	65	74	77	—
27	90	83	83	—	58	61	63	—	64	73	76	—
28	88	84	80	—	58	62	61	—	67	73	77	—
29	86	84	83	—	58	62	64	—	67	74	72	—
30	87	85	82	—	58	63	62	—	68	74	76	—
$\bar{x} \pm \sigma_{\bar{x}}$	86 ±1	83 ±1	82 ±2	84 ±2	57 ±1	61 ±1	62 ±1	58 ±2	66 ±1	73 ±1	76 ±2	70 ±2

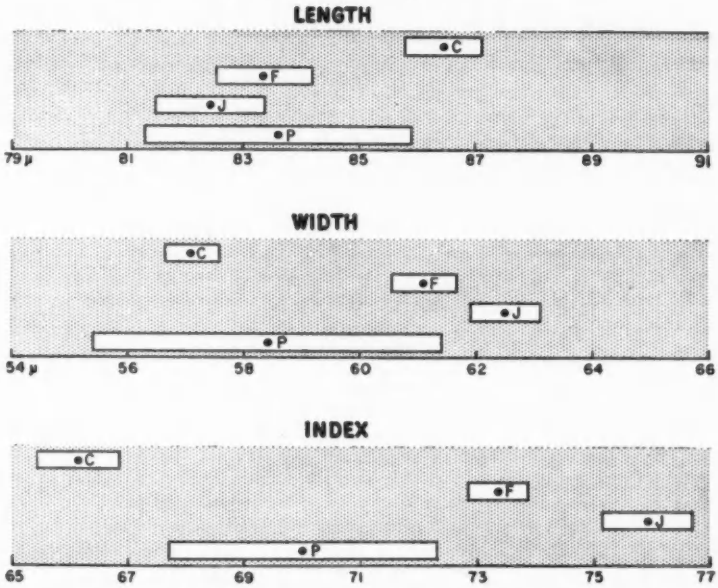

FIG. 2. Diagram showing the 99 per cent confidence interval of the mean of the means of 50 eggs of various *S. japonicum* strains from 30 mice. The central dot in each rectangle represents the mean of the means. C means the Chinese strain, F the Formosan, J the Japanese, and P the Phillippine.

the three different strains reported above shows that the daily variation of the size and shape of the eggs does not affect the pattern of the interstrain variation.

Confidence interval for the true means of the means

As the mean of the means of length, width and index of the eggs of *S. japonicum* from a number of hosts may be of importance in differentiating the strains, it is of interest to know the confidence interval of the true means of the measurements of the eggs from a host-species infected with various strains of the parasite. To determine this, 30 mice were infected with each of the three strains, Chinese, Formosan and Japanese; and seven were infected with the Philippine strain. A mean of the length, width and index of 50 mature eggs was obtained from each host-individual. The mean of the 30 means of 50 eggs from 30 mice was obtained (Table 3). Using the method for small samples, the 99 per cent confidence levels for the true mean of the means was determined. The length of the eggs ranged from 85.8 to 87.2 μ in the Chinese strain, 82.5 to 84.2 μ in the Formosan, 81.5 to 83.4 μ in the Japanese, and 81.3 to 85.9 μ for the Philippine strain. The width ranged from 56.6 to 57.6 μ in the Chinese strain, 60.6 to 61.7 μ in the Formosan, 61.9 to 63.1 μ in the Japanese, and 55.4 to 61.4 μ in the Philippine strain. The index ranged from 65.4 to 66.9 in the Chinese strain, 72.8 to 73.9 in the Formosan, 75.2 to 76.7 in the Japanese, and 67.7 to 72.3 in the Philippine strain.

The confidence intervals of the mean of the means of the length or width of

the eggs overlapped to a certain extent between certain strains, i.e., the length of the Formosan, Japanese, and Philippine strains, and the Chinese and Philippine strains; the width of the Chinese and Phillippine strains, and the Formosan and Philippine strains. On the other hand, the confidence interval for the index of the four strains did not overlap (Fig. 2).

DISCUSSION

In their study of the size and shape of the mature eggs of *S. japonicum*, Faust and Meleney (1924) mentioned that the size of the egg could be influenced by the permeability of the shell and by the amount of secretory and excretory products deposited between the miracidium and the egg shell. This was kept to a minimum in the present experiments by mounting all the eggs in normal saline. In comparing sizes of eggs, Faust and Meleney used the products obtained by multiplying the length of each egg by the square of the width, i.e., the actual cubic contents of the eggs. As the egg of this parasite was slightly flattened dorso-ventrally, we deemed it better to use the measurements of length, width and index for comparison. It should be emphasized that the proper orientation of the egg of *S. japonicum* before measurement is very important. The calculation of the index used in the present study as a basis for comparison is believed to reduce also the effect of the size variation caused by the deposition of the secretory and excretory products by the miracidium.

In the study of the confidence interval for the true mean of means of the eggs of different strains, the merit of the index of the eggs was well demonstrated. As the differences in size and shape of the eggs of different strains can only be shown by the measurement of a considerable number of eggs from a number of individuals and by comparing the means of these measurements, a positive identification of the strain cannot be made on an individual egg or by the measuring of a number of eggs from a single host-individual. As the size and shape of the eggs are to a certain extent influenced by the species of the host, the comparison of eggs between different strains has to be made on the eggs from the same host-species.

On the basis of egg measurements, Alves (1949) reported that there are two distinct strains of *S. haematobium* in Africa. The eggs of the West African Sudanese strain are closely related to those of *S. mattheei* while the eggs of the Rhodesian strain are not. Amberson and Schwarz (1953) reported that there was a higher percentage of short, broad eggs among those from *S. haematobium* in Egypt than from this parasite in South East and South Africa. On the basis of this and of the difference in the snail host, they went so far as to create a number of subspecies for *S. haematobium* in Africa. A new variety of *S. mansoni*, i.e., *S. mansoni* var. *rodentorum*, has been described from wild rodents in the Belgian Congo mainly on the minor morphological differences of the eggs (Schwetz, 1953). The present study indicates that the four strains of *S. japonicum* show similar differences.

As mentioned in another paper (Hsü and Hsü, 1957), we believe that there is no indication that the Chinese, Japanese and Philippine strains, all anthropophilic, are more closely related to one another than to the Formosan, the zoophilic strain. The results of the present study indicate that insofar as the size and shape of eggs are concerned, all four strains are distinct and can be

distinguished from one another. Although all four strains may have originated from one ancestral stem, each strain has developed its own characteristics.

SUMMARY

The measurements of the eggs of *S. japonicum* from dogs, cats, hamsters and albino mice infected with the Chinese, Formosan, Japanese and Philippine strains were compared. The size and shape of the eggs showed significant differences even between host-individuals of the same species infected with the same strain of the parasite. Between strains, if the mean of the measurements of a number of eggs from a host-individual was obtained and the collective means from a number of host-individuals infected with one strain were compared with those of the other strains, significant interstrain differences were found in the length, width and especially in the index of the eggs. The confidence intervals of the mean of the means of the index of the eggs were very distinct, i.e., there was no overlapping among the four strains. The variations of the eggs between host-species were significant in certain cases. Accordingly the interstrain comparison of the size and shape of the eggs of this parasite should be made for the eggs from the same host-species.

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REFERENCE

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BOOKS RECEIVED

The Story of Peptic Ulcer, conceived by Richard D. Ronkin; characterized by Raymond Keith Hellier, 71 pages, illustrated. Philadelphia, W. B. Saunders Co., 1957. $2.25.

The Appraisal of Applicants to Medical Schools. Report of the Fourth Teaching Institute Association of American Medical Colleges, Colorado Springs, Colorado, November 7-10, 1956. Edited by Helen Hofer Gee and John T. Cowles. 228 pages, illustrated. Evanston Illinois, Association of American Medical Colleges, 1957. $3.00 cloth, $2.00 paper.

BOOK REVIEWS

World-Atlas of Epidemic Diseases, Edited by Ernst Rodenwaldt and Helmut J. Jusatz, under the Sponsorship of the Heidelberger Akademie der Wissenschaften. First of four issues in the second part of the series. 54 pp. 10 maps. Hamburg: Falk-Verlag, 1956.

The first instalment of the second part of the Atlas of Epidemic Diseases is made up of 10 excellently executed colored maps, each of which is accompanied by additional small black- and white maps (inserted mostly on the reverse side of the colored maps) and explanatory texts in German and English, and deals on the one hand with disease situations in Europe (relapsing fever in 1922; epidemic myalgia (Bornholm disease), 1931–52; smallpox in Europe, 1919–1948; rabies in Central Europe, 1947–53), on the other hand with the incidence of certain diseases in non-European countries, namely, bacillary dysentery, trachoma and sleeping sickness in Africa; Chagas' disease in Central and South America. A tenth map illustrates the distribution of the population in the United States and adjoining territories in 1950/51. It is gratifying to note that most of the valuable discussions of each of the diseases or conditions illustrated in the maps have been more or less fully translated from the German into English. Unfortunately, however, this does not hold true of the article dealing with the occurrence of bacillary dysentery (caused by *S. dysenteriae*) in Africa during the period of 1900–1950, the particularly interesting introduction to which has received scanty attention in the English translation. The most important statements made in the German original are that (a) bacillary dysentery is by no means only a disease of the temperate zones, but is also more frequent than the amebic type in the tropics and is characterized there by a mortality higher than that of the amebic form; and (b) the disease is particularly prone to attack persons such as merchants, soldiers, officials or tourists, who have newly arrived, but is by no means absent among the resident white population, whereas (c) it is rampant among the native population as a disease of the earliest periods of life, being thus one of the main reasons for the high mortality of infants and small children. Nevertheless, for various reasons, bacillary dysentery may also assume epidemic proportions among the adult native population. Among the other subjects dealt with in this instalment of the Atlas undoubtedly the most interesting for American readers are the two maps showing the incidence of Chagas' disease in Central and South America and the accompanying exhaustive articles by E. Dias, Instituto Oswaldo Cruz, Rio de Janeiro. Generally speaking, the information conveyed by the maps and the adjoined texts in the issue of the Atlas under review are valuable as an introduction to the knowledge on the incidence and epidemiology of the diseases which have received attention.

R. Pollitzer

World-Atlas of Epidemic Diseases, Edited by Ernst Rodenwaldt and Helmut J. Jusatz, under the Sponsorship of the Heidelberger Akademie der Wissenschaften. Second of four issues in the second part of the series. 38 pp., 10 maps. Hamburg: Falk-Verlag, 1956.

The instalment of the World-Atlas of Epidemic Diseases under review is of special interest for students of tropical diseases, since, besides dealing with some disease conditions in Europe (paratyphoid A; diphtheria; trench fever) and with the population distribution in the Mediterranean area and in Africa in 1950, it devotes attention to (a) yellow fever in Africa, 1939–1952; (b) dengue epidemics in the Mediterranean area (1800–1950) and the distribution of its vector *Aedes aegypti*; (c) geographical distribution of leishmaniasis and phlebotomus vectors in Africa, 1906–1952; and (d) in a particularly well documented article accompanying the corresponding map with the

distribution of filarial infections (filariasis and dracunculosis) in man in Africa with special reference to the position since 1939.

Discussing the incidence and epidemiology of yellow fever, Höring & Höring come to the conclusions that the Central-African jungle is the original home of this virus infection and that the disease was imported from Africa through slave transports into Central America and only afterwards became entrenched among the monkeys of the South American equatorial forest regions. The main reasons why Asia has remained unscathed thus far and is likely to remain free from yellow fever are (1) the presence of geographical barriers, consisting of mountainous and savanna regions as well as the wide Indian Ocean, which separate off the Central-African endemic areas to the east; and (2) the absence of any large tropical jungles in Asia, which, as the authors put it, "could be compared even remotely with those of Central Africa and the Amazon region."

It is gratifying to note that the authors bestow due praise on the yellow fever research work recently undertaken in Africa under the auspices of the Rockefeller Foundation, which led inter alia to the discovery of an important additional role played in the transmission of the infection by *Aedes africanus Theobaldi*.

Though paying main attention to the problems of dengue in the Mediterranean area, Ulmann also refers to the world-wide occurrence of this disease. He notes in this connexion that, though undoubtedly *Aedes aegypti* is the sole vector of the infection in the countries round the Mediterranean, probably other *Aedes* species play a role in other parts of the world, e.g., *A. argenteus* in Texas.

It is regrettable that, as is also the case with some of the other articles of this instalment, the English translation of that dealing with dengue is not felicitous, and that, therefore, less benefit can be derived from it than from the German original. In fact, one statement made in the English translation regarding the role of the vectors and the biology of the dengue mosquitoes says the opposite of what is correctly stated in the German text.

R. POLLITZER

World-Atlas of Epidemic Diseases, edited by ERNST RODENWALDT AND HELMUT J. JUSATZ, under the sponsorship of the Heidelberger Akademie der Wissenschaften. Third of four issues in the second part of the series. 50 pp. 10 maps. Hamburg: Falk-Verlag, 1955.

The present instalment of the World-Atlas of Epidemic Diseases contains excellently executed colored maps illustrating (1) the global morbidity of scarlet fever, 1919–1953; (2) types of courses and lethality of scarlet fever 1928–1938 and 1939–1945; (3) plague in Europe 1899–1952; (4) plague in Africa; (5) tularemia in Central Europe 1933–1953; (6) louse-borne relapsing fever 1800–1950 (global incidence); (7) tick-borne relapsing fever 1905–1950 (global map); (8) Q fever in Europe, North Africa and Asia minor 1940–1954; (9) global incidence of amebic dysentery and *E. histolytica* 1903–1953; (10) global distribution of ascariasis 1907–1950.

Each of these maps is accompanied by a carefully written and adequately documented text printed on separate sheets of the same size as the maps, containing graphs and tables. Moreover the reverse sides of the colored maps have been utilized in various ways. Thus that on scarlet fever morbidity has on its reverse side a black and white map with accompanying table showing Dick-test results in relation to morbidity and lethality, while in that on scarlet fever lethality tables and graphs as well as a smaller map showing the development of scarlet fever morbidity and lethality in Europe 1920–1945 will be found. The reverse side of the plague map contains, besides a reference list and some graphs, a small map illustrating the spread of this infection in the Eastern Mediterranean area during the period 1913–1930 and again in 1945. The great value of the map on tularemia in Central Europe is further enhanced by a second map inserted on the reverse side, which illustrates details of an outbreak in the Steigerwald area, Southwest Germany, during the period 1949–1952. Interesting supplementary maps have been also provided on the reverse sides of most other maps, among which those on tick-borne relapsing fever in two areas of the United States will specially appeal to readers in this country. The reverse side of the ascariasis map brings, in place of a supplementary map, comprehensive statistics on the global incidence of this infestation arranged according to the places of observation.

Even this bare enumeration will show that this part of the atlas contains a great deal of data culled from numerous and often by no means easily accessible sources. Besides thus serving as a source of reliable prima facie information on the diseases dealt with, it ought to be of value for didactic purposes.

R. POLLITZER

World-Atlas of Epidemic Diseases, Edited by ERNST RODENWALDT AND HELMUT J. JUSATZ, under the Sponsorship of the Heidelberger Akademie der Wissenschaften. Fourth of four issues in the second part of the series. 26 pp., 10 maps. Hamburg, Falk-Verlag, 1956.

Of the 10 maps in the 4th instalment of the second part of the Atlas of infectious diseases, six illustrate various aspects of the climatology of Africa, namely (a) mean actual January temperature; (b) mean actual July temperature; (c) mean annual precipitation; (d) rainy seasons; (e) dry seasons; (f) mean annual thermic sultriness values. There can be no doubt that these beautifully executed maps, which are accompanied by a comprehensive article on the climate of Africa by Professor Knoch, will prove valuable for all who wish to study the incidence and epidemiology of diseases, specially of tropical diseases, in Africa.

The four remaining maps of the instalment deal respectively with (i) leprosy in Africa, 1953; (ii) poliomyelitis in Africa, 1919–1954; (iii) smallpox in Africa, 1920–1953; and (iv) malaria in Central Africa, of which the first and the fourth are of particular interest for students of tropical diseases.

Commenting in the text of the Atlas upon observations on leprosy in Africa during the period 1945–1954, Littann points to the divergent views held regarding the origin of this disease: while some authors maintain that it spread in epidemic form from Central Africa to the rest of the world, many writers point to the influence exerted on the spread of the infection in the African continent from outside, particularly from India. Certain it is that Africa played at least an important role as a relay station in the spread of leprosy, being responsible for early invasions of Europe since the time of Alexander the Great and also of Central and South America, effected according to Littann at the beginning of the 16th century through slave transport.

That Africa played such an ominous role is not surprising since, as Littann puts it, "a zone with the worst endemic occurrence of leprosy (over 15 per thousand) and the highest infection rate in the world crosses like a belt from the West African Coast through Central Africa." In this continent, as also in Europe, leprosy has recently become most prevalent among the rural populations, while in the urban centers the incidence of the infection appears to have decreased pari passu with an increased tuberculosis incidence.

The subject of malaria in the Netherlands and in West Germany during the period 1900–1950 is discussed exhaustively and adequately in articles by De Jong and by Steiniger. In spite of the availability of potent drugs and of the modern insecticides, De Jong finds it impossible to assert that malaria does not remain potentially dangerous for the Netherlands. Even though malaria has practically disappeared from Western Germany for the time being, according to Steiniger "its absence is not an absolute one but rather due to continued measures against it which in part are carried out unconsciously."

R. POLLITZER

Wege der Seuchen—Lebensgemeinshaft, Kultur, Boden und Klima als Grundlage von Epidemien. Unter Berücksichtigung der Tropenkrankheiten dargestellt, by DR. PHIL. ET MED. ERICH MARTINI, Professor am Institut für Schiffs- und Tropenkrankheiten L. R. 3d ed., revised and enlarged. 203 pp. Stuttgart, Ferdinand Enke Verlag, 1955. Paper DM 19.; bound DM 21.80.

The well-known parasitologist at the Nocht Institute for Schiffs- und Tropenkrankheiten at Hamburg has revised his "Pathways of Epidemics," which first appeared in 1936 as an amplified series of lectures. According to the introduction, the treatise is primarily intended to familiarize the practicing physician, biologist and interested layman with the complex interrelation of contagious and parasitic diseases with human culture, soil and climate. By means of examples, most of them chosen from the vast experience of the author, principally in the field of tropical medicine, the facts of modern epidemiology are well covered. Sometimes, because of the complex style and the choice of such words as "*Heben* einer Seuche" the presentation is quite involved and certainly would be difficult for a reader not familiar with the subject to follow. The hygienist will profit greatly by studying the chapter on reservoir hosts and zoonoses in general. The complexities of heterogenous infection chains, particularly those in which arthropods form links, are illustrated by some examples not generally known, such as the poultry disease induced by *Prosthogonimas pellicudus* (p. 47). The existence of "natural enzootic foci of zoonoses," which are determined by topography, climate, vegetation and other factors are mentioned, but the idea is not carried to one important conclusion: when these factors are found assembled, the assembly can serve as an indicator of the potential presence of certain diseases. In the chapter on soil and epidemics the reader misses the subjects of tetanus, anthrax and botulism. The reservoir problem of plague in the rodents of the wilds (p. 135) refers to the appearance of this disease in Oregon and Montana. The author states that the principal wheat areas of the American Continent are now open and predicts that the progress of plague to the Atlantic Coast can hardly be prevented. This particular crystal ball reading is not supported by facts. References are few, and one wonders about the source of some of the information. For example, on page 171 it is incorrectly stated that *Amblyoma* ticks are the transmitters of heart water to horses, when in reality the virus

infects sheep, goats and cattle. A reader who has already mastered German and is willing to study, rather than merely read, the content of the treatise will derive great benefit and pleasure. He will broaden his outlook on parasitology and epidemiology.

K. F. MEYER

Annual Review of Entomology by EDWARD A. STEINHAUS, Editor AND RAY F. SMITH, Associate Editor, University of California. Volume 2, first edition, 407 pp., Palo Alto, California: 1957. Annual Reviews, Inc. $7.00.

This second volume of reviews of selected entomological subjects maintains the high standards and objectives of the first volume. Leading workers from six countries have contributed 20 chapters and each is a pertinent review and interpretation of current worldwide studies in its field. These contributions are to be recommended to both the professional worker and student in entomology. The reviewer would draw the attention of readers of this journal to ten chapters. Of specific interest are "Recent advances in veterinary entomology" by Lindquist and Knipling, and "Transmission of disease agents by phlebotomine sand flies" by Adler and Theodor.

The chapter on veterinary entomology is largely devoted to biting flies, bot flies and blow flies. There are short sections on lice and radioisotopes in research. Rather detailed consideration is given to systemic insecticides and screwworm control with sexually sterile males. The subjects of ticks, mites and houseflies are not reconsidered, as they were included in Volume 1. It is perhaps unfortunate that a more widespread coverage was not given to other arthropod groups and to a more complete coverage of arthropod-transmitted disease agents of animals. The extensive current literature on myxomatosis in Australia and Europe might well be considered in the field of veterinary entomology but is not mentioned.

The chapter on sand flies offers a timely and well-done review of the transmission of pathogens by these vectors. Pertinent data on bionomics are provided as a background interspersed into the detailed consideration of the leishmaniases, bartonellosis, and sand fly fever. One wishes the authors had considered possible differences in vectors for the Sicilian and Naples types of virus, and might differ with their unequivocal interpretation that "epidemiological evidence points to transovarian transmission in *P. papatasi*."

The remaining chapters of possible direct application to studies in tropical medicine and hygiene are devoted to Digestion in insects, Cytogenetics and systematic entomology, Dynamics of animal populations, the Synaptic approach to studies of insects and climates, Genetics of insect resistance to chemicals, the Mode of action of insecticides exclusive of organic phosphorus compounds, Chemistry and mode of action of organophosphorus insecticides and Aerial application of insecticides. An additional ten chapters represent essential contributions on physiology, morphology, taxonomy or agricultural aspects of entomology.

W. C. REEVES

Jordan's Tropical Hygiene and Sanitation. Bailliere's Elementary Tropical Handbooks, by W. WILKIE, Instructor in Hygiene, School of Hygiene, Uganda. 3rd ed., 408 pp., illustrated. Baltimore, The Williams and Wilkins Co., 1956. $5.00.

This third edition of a well known handbook on tropical sanitation has had considerable revision and additions, especially in the chapters on ventilation, water and communicable diseases. Chapter III (73 pages) on building construction would appear to an American to be superfluous, as this work would be handled by a building inspector under our practice. Furthermore, much of the material therein appears to be rather obsolete in view of modern knowledge of construction of cool houses in hot climates. Under "Communicable Diseases" we note rather longer isolation periods for the acute exanthemata than are necessary, and for typhus control (pg. 284) an emphasis on old methods rather than emphasis on DDT delousing. However later, on pp. 326–327, the use of DDT in delousing is emphasized. Better correlation and cross-referencing of relevant sections would be an improvement. Some typographical errors (mosquito species named on pg. 334 for example) were noted.

Although obviously a manual of tropical sanitation, the book appears to be designed to meet the requirements for passing the Royal Sanitary Institute examinations for sanitary inspectors in the tropics, and also for use in teaching native inspectors with a limited knowledge of English. Since its first publication in 1939 this has been a useful compendium of information on tropical sanitation. Its value is not limited to Africa, though some of its dicta will need modification in tropical America and Asia.

HAROLD F. GRAY

Medical Parasitology for Medical Students and Practicing Physicians, by WILLIAM G. SAWITZ, M.D., Professor of Parasitology, Associate in Medicine, The Jefferson Medical College of Philadelphia. 2nd edition. 342 pp., illustrated. New York, McGraw-Hill Book Company, Inc., 1956. $6.00.

This book is intended for medical students and

physicians. It is written in a clear concise manner, with emphasis placed on the medical aspects of parasitic diseases, while only such zoological data are presented as the author believes will make a contribution to a specific diagnosis of the disease under consideration, and to an understanding of its pathogenesis and epidemiology.

The animal parasites are grouped according to their phylogenetic relationships under the section headings of Protozoa, Helminths and Arthropods. Information is provided on the life cycle and morphology of the agent or vector, and on the clinical manifestations, diagnosis, treatment and prevention of the disease caused.

A new section entitled "Synopsis" appears in this second edition. Written partially in tabular form, it summarizes basic and fundamental points necessary for an understanding of parasitism and parasitic diseases, including resistance and immunity, and hematology in parasitic diseases.

The section on treatment has been revised considerably to bring it up to date. Useful drugs, their synonyms and/or their chemical names are listed for each disease, and information on dosage, efficacy, toxicity and contraindications is given. The sections on "Techniques for Laboratory Diagnosis" and "Vocabulary of Technical Terms" have been changed slightly by eliminating some material of the first edition and by the addition of new material.

This book is forty-some pages longer than the first edition. Material on clinical manifestations and prevention has been amplified. There are several pages of additional material on the protozoa, and information on visceral larva migrans is added to this edition. Several inaccuracies occurring in the first edition have been eliminated, but the synoptic style of writing employed, with the resulting authoritative statements on some points, has made it difficult to prevent the appearance of some minor inaccuracies in this second edition. Several of the figures have been revised and improved. A few new figures have been added, and some that appeared in the first edition have been omitted.

This is a practical small book that presents in a commendable way important basic knowledge on a very extensive subject. It should be very useful to students of medicine and medical technology and to physicians who have only a very limited time for the study of this broad and important subject. Those who use the book should not harbor the delusion that it is an adequate substitute for the more comprehensive texts on the subject, but rather should consider that it satisfies a much-felt need for a smaller book on the subject which can be more adequately studied in the time now allotted to parasitic diseases in the curricula of the majority of our medical schools.

WM. HUGH HEADLEE

Practical Dermatology, by SAMUEL M. PECK, B.S., M.D., Dermatologist to the Mount Sinai Hospital, New York: Associate Clinical Professor of Dermatology, Columbia University; AND LAURENCE L. PALITZ, M.D., Ph.D., Attending Dermatologist, Long Island Jewish Hospital. 1st edition, 380 pages illustrated. New York, McGraw-Hill Books Company, Inc., 1956. $7.00.

Drs. Peck and Palitz have succeeded in presenting a wealth of modern practical diagnostic and therapeutic measures for the general practitioner to use in selected common dermatoses. Since the authors have a vast experience in practice, and since the senior author has been at his profession for well over 25 years, this permits some conclusions based on personal observations, conclusions which may not all be in accord with orthodox teaching or majority opinion. And indeed the authors are aware of this, for they state in their preface: "This will be a personal book. Our own personal belief concerning etiology and pathologic-physiology will be presented." It is therefore not astonishing that there will be certain areas of sharp disagreement between the authors and some of their colleagues.

Among the possible areas for disagreement are: the minimizing of the value of palpation as a diagnostic aid (p. 24); the statement that a new complement-fixation test is of practical value for the diagnosis of candida infections of the skin (p. 35); the degree of confidence expressed in methods for the treatment of recurrent herpes simplex (p. 50); the statement that Jadassohn and Kingery (sic) had demonstrated the virus etiology of warts (p. 55); the systemic use of calcium lactate or hydrochloric acid and local use of local peeling paste in the management of acne rosacea without any direct mention of the value of the internal administration of modern wide spectrum antibiotics for the acne element of the eruption (pp 105 and 106). The authors' classification of the "eczemas" of infancy and childhood (pp 134–140) seems not only unorthodox but quite unclear—at least to this reviewer. Moreover it does not appear to add anything likely to prove useful to the non-dermatologic practitioner. It is a great pity that most of the illustrations are very poor indeed. With a few notable exceptions the photographs add nothing of value or of style.

On the other hand good features are present in great number. They include for example the section on industrial dermatoses (pp 107–120) and the "Suggested Form for Reporting an Industrial Dermatitis"; the discussion of the Skin Manifes-

tations of Vascular Disturbances (pp 187–202) with one of the most complete Tables of Classification of these changes, namely the one by Peck, Rosenthal and Erf (pp 194–197) and the chapter on the Common Pigmentary Disturbances of the Skin (pp 233–240).

All in all no reader or user of this book could fail to gain much practical information, some of which is not to be found in any other short text.

Soybeans for Health, Longevity, and Economy, by PHILIP S. CHEN, PH.D., Professor of Chemistry, Atlantic Union College, with the assistance of Helen D. Chen, M.A., National Science Foundation Fellow, Cornell University. 1st edition, 241 pages, illustrated. South Lancaster, Mass. The Chemical Elements, 1956. $3.00.

Soybeans have been promoted for many years by the U. S. Dept. of Agriculture, the Bureau of Home Economics and Human Nutrition, various state agricultural extension services and numerous others as an economical food of high protein and oil content which has many industrial uses.

This book contains a wide variety of information on the soybean, an attractive format, good quality paper, and fair illustrations. Approximately one quarter is devoted to a discussion of the nutritive value of the soybean, about one quarter to soy products, and the rest to soybean culture, preservation, and recipes. Many of the recipes are selected from state agricultural extension and U.S.D.A. bulletins. The appendix contains a list of manufacturers and handlers of soy foods, and some selected references of which, excepting the publications of The American Soybean Association, only one is later than 1950.

One of the important needs for feeding the world's population is a source of high quality protein at a low cost. The section of this book on nutritive value of the soybean is the least satisfactory. Dr. Chen seems divided between his knowledge of the subject when he states on page 12 that "while the protein efficiency and biologic value of the soybean is not too high when used alone, these values are greatly increased when it is used to supplement wheat flour," and his enthusiasm which leads him to say three pages later that "the protein of the soybean is not only of the same nutritive efficiency as that of meat, eggs, and milk, but it also has other advantages." Although the protein quality of soybeans is better than that of other legumes and some cereal grains, it is not equal to that of milk, eggs, and meat. Nevertheless, after reading this section, the reader is apt to conclude that they are, as is evidenced by the statement on the jacket, "It is the only vegetable food that yields a complete protein comparable in quality to that of milk, eggs, and meat." Soybean protein is deficient in methionine and so is not a complete protein. But it does contain lysine, an amino acid in which most cereal grains are deficient, and so is a good supplement for these foods. The table on the essential amino acid content of some foods is misleading as it is the balance or ratio of amino acids present, not the total amount, which affects protein quality. The discussion on protein quality includes little work done since 1947.

The chapter on "Soybeans and Disease" has poorly selected material, especially on cholesterol and coronary disease. One page out of the 57 pages on nutritive value is devoted to a letter to the editor of "The Soybean Digest" in 1944 on the merits of soybean oil in relieving constipation.

B. M. KENNEDY

NOTICE TO CONTRIBUTORS

The Journal welcomes papers which contribute to the purpose of the American Society of Tropical Medicine and Hygiene. This is the advancement of knowledge of tropical and subtropical medicine and hygiene through investigation, prevention, treatment and control of diseases and deficiencies. It draws upon medicine, biology, public health, nursing, engineering and allied fields of research and application.

The American Journal of Tropical Medicine and Hygiene combines the American Journal of Tropical Medicine and the Journal of the National Malaria Society and therefore has a special concern with the investigation, therapy and control of malaria in all parts of the world, as well as a broad interest in general parasitology and arthropod-borne diseases.

Papers are ordinarily published in the order of reception, which will entail a certain delay in their appearance because of the contractual limitation on the number of journal pages available in any year. Accepted articles which raise no question of scientific priority but for which immediate publication seems important will be inserted in the next available issue of the journal if the author or sponsoring institution will pay the cost, estimate of which may be obtained from the editor. The insertion of such articles does not affect publication of other manuscripts awaiting their turn, since the inserted articles constitute additional pages in the volume without additional cost to the subscriber.

Publication will be facilitated if the following suggestions are followed:

1. *Manuscripts* should be typewritten in *English* using *double spacing* on *one side* only of 8½ x 11 inch paper and submitted *in duplicate*. Double-spacing should be used throughout the manuscript including the list of references, footnotes, and quotations.

2. The author should supply, on a separate sheet, a short title not over 50 letters for use as a running head. Also on separate sheets type (a) the authors' names and affiliations; (b) tabular matter; (c) case histories; (d) quotations; (e) formulas and other subsidiary matter in the text; (f) footnotes; (g) references; (h) legends for illustrations; (i) displayed formulas occupying more than 1 line of type, even if using the same size type as the main text.

3. Legends should be separate and not attached to, or written on, the illustration copy.

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7. *Figures* and *photographs* should be protected with cardboard to avoid creasing in the mails. Only glossy prints can be used. Line drawings and graphs should be made with India ink and submitted on white (not cross-section) paper. Colored illustrations can be reproduced only at the expense of the author.

8. *References* should be cited in text by author and date, and the list should be in alphabetical order according to authorship, with the date following the author's name, e.g.:

REED, L. J., AND MUENCH, H., 1938. A simple method of estimating fifty per cent endpoints, *Am. J. Hyg.* **27**: 493–497.

9. *Proprietary names* of drugs or chemicals registered by commercial pharmaceutical firms may not be used in the text if there is a generic name available in the U. S. Pharmacopoeia, New and Nonofficial Remedies of the A. M. A., or the latest editions of standard medical dictionaries. In certain cases where the generic name is relatively unknown, the registered name may follow it in parentheses in the title, at the first citation in the text, and in the conclusions.

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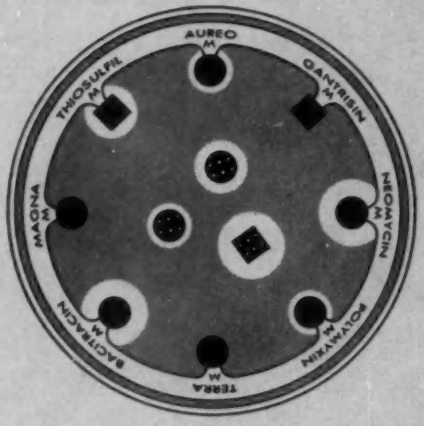

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Bacto - Sensitivity Disks are prepared in three concentrations of each agent; colored and marked for quick and accurate identification.

AVAILABLE ON REQUEST

Bulletin No. 152, describing Bacto - Unidisks
Bulletin No. 146, Microbial Sensitivity Testing
Report sheets for reporting Microbial Sensitivities

DIFCO LABORATORIES

DETROIT 1, MICHIGAN